Transplantation and the Nature of the Immune Self

by G. V. Ramesh Prasad

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Arts

in

Philosophy

Waterloo, Ontario, Canada 2014

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

There are many theories regarding the nature of the immune self. However, existing theories have not accounted for the clinical experience of transplantation. Kidney transplantation provides a unique opportunity to examine the nature of the immune self because the immune system is deliberately engaged at a known point in time. The self-nonself theory provides a dichotomy that is very useful for pre-transplant preparation but it fails to provide sufficient explanation for the diversity of post-transplant phenomena. The liquid self hypothesis provides a more adequate explanation for the variation in post-transplant outcomes because it accounts for the spatiotemporal evolution of the immune self in response to the environment. The immune self is always changing. The switch between self and nonself status for all antigens is the essence of the continuing change in the immune self. The success of transplantation is determined by how well the immune self adapts to the challenge that a transplant imposes.

Acknowledgements

I would first like to thank the University of Waterloo for providing a mid-career practicing physician such as me the opportunity to expand his horizons by pursuing advanced training in Philosophy. I do not think such an endeavor would have been possible anywhere else in the world.

I would also like to thank my supervisor, Dr. Paul Thagard, for so willingly accepting me as his graduate student. His great enthusiasm for the project has made my educational experience very special. I am very grateful for his insightful comments and constant encouragement, and I sometimes wonder what my career would have been like today if I had met him twenty years ago.

I wish to convey my appreciation to my thesis readers, Dr. Christopher Lowry and Dr. Steven Weinstein, for their helpful and timely suggestions. I would also like to thank all the graduate students, staff, and faculty in the Department of Philosophy at the University of Waterloo. It was because of all of them that I had a lot of fun being a graduate student again.

Finally, I express sincere gratitude to my colleagues in the Division of Nephrology, Department of Medicine, St. Michael's Hospital, Toronto, for allowing me the scheduling flexibility to pursue this dream. In particular, I would like to thank Dr. Jeffrey Zaltzman and Dr. Kamel Kamel.

Dedication

This thesis is dedicated to my parents, who were my first teachers and continue to be my greatest source of inspiration. I also dedicate it to my three children, for giving me something to smile about each day.

Many of the clinical observations that ultimately provided a significant amount of material for this thesis resulted from my interactions with a large number of patients over the years. I express my thanks by also dedicating this thesis to them.

Table of Contents

List of Figures vii

List of Tables viii

Chapter 1 Introduction

1.1 A Philosophical Approach to Transplantation 1

1.2 An Introduction to the Immune Self 4

Chapter 2 Transplantation and Engagement of the Immune Self

2.1 The Immune Response 12

2.2 Acute and Chronic Rejection 15

2.3 Theories of the Immune Self 19

2.4 The Transplant Perspective of the Immune Self 31

Chapter 3 Transplantation and Complications of the Immune Self

3.1 The Transplant in an Alien Environment 48

3.2 Transplant Complications from the Immune Self Perspective 53

Chapter 4 Summary and Conclusion

4.1 Summary 64

4.2 Conclusion 66

References 68

List of Figures

Figure 1 The immune response 13

List of Tables

Table 1	Major causes of chronic rejection (chronic allograft nephropathy, interstitial fibrosis/tubular atrophy)	18
Table 2	Common side effects of anti-rejection medication	49

List of Abbreviations

APC	antigen-presenting cell(s)
BKV	BK virus
BP	blood pressure
CKD	chronic kidney disease
CMV	cytomegalovirus
DNA	deoxyribonucleic acid
EBV	Epstein Barr virus
ESKD	end-stage kidney disease
GVHD	graft-versus-host disease
HLA	human leukocyte antigen
HSV	herpes simplex virus
IF/TA	interstitial fibrosis/tubular atrophy
MHC	major histocompatibility complex
NK	natural killer
TCR	T cell receptor
WBC	white blood cell

Chapter 1

Introduction

1.1 A Philosophical Approach to Transplantation

End-stage kidney disease (ESKD) is a fatal condition if it is left untreated. Kidney transplantation is widely considered to be the treatment of choice for patients with ESKD largely because it provides the maximum possible replacement of lost kidney function for patients. Both patient quality of life and long-term survival are significantly improved (Wolfe 1999).

Kidney transplantation also represents a good example of a successful multi-disciplinary medical enterprise. Many professionals are involved in the different stages of the transplant process. Physicians caring for the patient prior to the transplant strive to keep the patient as healthy as possible, so that they may withstand the surgical operation without the serious complications that could then make the post-transplant course much more difficult. Transplant administrators ensure a fair and equitable distribution of these scarce organs, and by doing so maintain society's trust in the system. They are responsible for the smooth flow of vital information to all those involved in the transplant process. Transplant immunologists ensure that the kidney donor and potential recipient are as compatible with each other as is reasonable, so the kidney will not be rejected. Thus, they help to select the most appropriate donor for a given recipient. Transplant surgeons with their great technical skill perform the operation with as little disruption to the bodily integrity of the recipient and the living donor as possible. Transplant physicians manipulate the immune system, titrating and adjusting the dose of various immunosuppressive or "anti-rejection" medications, and sometimes switch these medications to others altogether (Meier-Kriesche 2006). Transplant physicians attempt to achieve a balance for their patients between preventing rejection from too little medication

on the one hand, and minimizing side effects from too much medication exposure on the other. Over many years, they also actively prevent, diagnose, and manage a variety of medical conditions that can have an impact on transplant performance. Anti-rejection medications in turn are developed and rigorously tested for safety and efficacy by biochemists, pharmacologists, and clinical trialists, sometimes for many years before they are first used regularly. At every stage in this transplant process, other consultants from a diversity of medical specialties as well as allied health personnel (including nurses, pharmacists, laboratory technicians, dieticians, and social workers) are of great assistance in making and implementing decisions. The end product of this huge enterprise is a successful kidney transplant that leads to a healthy patient who can enjoy a vastly improved quality and quantity of life.

Throughout this vast transplant process, however, there has been no defined role for the transplant philosopher. For sure, important ethical conflicts arise on occasion and are handled by bioethicists. Examples of such ethical issues of concern include how to allocate scarce organs to patients on long waiting lists according to principles of justice, how to properly select living kidney donors so their health is not compromised, and how to manage deceased donors while respecting their expressed wishes and those of their family. Beyond these ethical situations, it is not too hard to imagine that various epistemological and metaphysical questions of interest can arise, for transplantation is after all a situation in which an important internal organ is permanently taken out of one human being and implanted in another human being. Philosophers have systematically approached some epistemological and metaphysical claims related to transplantation, but at least to my knowledge, there have not been similar attempts by a transplant professional. This thesis represents one such attempt by a transplant professional seeking to become a philosopher.

I have chosen for my thesis topic a description of what transplantation has to teach us about the nature of the “self”. The “self” is an oft-used yet ambiguous term most commonly employed in the psychology literature, although it originated in philosophy. One

particular aspect of the self is the so-called “immune self”, which is a term used in the immunology literature. While the molecular and cellular nature of this immune self profoundly interests immunologists, and perhaps some transplant professionals like me, its study also permits us to examine questions that are of primarily philosophical interest. Is there really such a thing as an “immune self”? Is there such a thing as an immune “nonself”? What is the role of this immune self? Is the immune self a constant entity, unchanging in time, or is the immune self constantly changing? Does the success or non-success of a transplant indicate how the immune self was able to adjust to and accommodate the transplant? Are the existing theories of the immune self adequate to explain what is commonly observed in the real world of clinical kidney transplantation? Here, I propose that clinical, “real world” kidney transplantation can provide us with some of the insights necessary to facilitate an informed approach to answer the last question listed, but perhaps also some of these other fundamental philosophical questions.

Scientific findings and philosophical speculation are generously mixed throughout this thesis on the nature of the immune self. I do so because I believe it is important to recognize that science and philosophy can each provide us with only partial answers and so at the same time must always leave us with tantalizing theory. In order to make further progress in our understanding of the nature of the immune self, science and philosophy need to extensively support each other. Science and philosophy fill in for each other’s deficiencies when necessary and at the same time constantly learn from each other. While others have harnessed this relationship in their investigations, I have uniquely added a fair dose of clinical medicine experience to this science-philosophy mixture. I am able to provide this thanks to my privileged position as a medical practitioner of transplantation, an intentional manipulator of the immune system in human beings, and a witness to the human self in transition.

1.2 An Introduction to the Immune Self

My hypothesis is that transplantation shows us that the immune self is ever changing, and the success of transplantation is determined by how well the immune self adapts to the challenge that a transplant imposes.

I propose that the self is constantly evolving as a response to the challenges it faces. Using the immune self as the representative of the self for this purpose, I am supposing that a transplant is the most significant challenge that an immune system can ever face, and is the challenge most likely to elicit a sudden change in the immune self. When framed as a scientific hypothesis, this seems simple enough.

Since the focus of my thesis is the nature of the immune *self*, it is important at the outset to distinguish the term "immune self" from other commonly used terms (including in this thesis) that contain the word 'immune". Understanding what these other terms mean is very important to understanding the immune self. These other terms are now briefly described.

The immune *system* refers to a group of specific organs including the bone marrow, thymus, spleen, and lymph nodes, along with a circulation system that consists of the lymphatic system and blood system. It also contains many types of white blood cells (WBC) (called immune cells), their molecules such as cytokines, and complement proteins (Pradeu 2012a). The immune system is a biological term used by immunologists and clinicians to denote the physical entities that perform the functions involved in immunity, such as the immune reaction and immune response.

The immune *reaction* refers to the biochemical interaction between an immune receptor and its ligand (Pradeu 2012a). This means any interaction between a receptor on any cell that is part of the immune system and what this receptor binds to, in order to carry

out its function. It is a normal phenomenon that is always occurring and is essential to health.

The immune *response* is the result of activation of an immune cell (Pradeu 2012a) typically leading to cell proliferation and differentiation. Depending on the type of cell involved, the end result of the immune response may be either destruction of the target, or inhibition of that destruction. Therefore, an immune response is preceded by an immune reaction, but not every immune reaction leads to an immune response (Pradeu 2012a).

The immune *self*, in contradistinction to the above terms, is very difficult to define. Multiple definitions and descriptions exist, as will soon become apparent. For our immediate purposes however, the immune self is what distinguishes between self and nonself components in the body and thereby "instructs" the immune system to carry out selective immune reactions and immune responses. The immune self probably consists of at least some of the cells and other components of the immune system, but this is not certain. It is more of a philosophical than biological term. Conceptually, the immune self underlies both the immune reaction and the immune response.

Since my focus is on immunity, we are not discussing whether there is a single, universal psychological self of whom we are all part, from where we all originated and to where we will all eventually return. This question is best left to experts in metaphysics and religion. We are also not discussing if a person goes through multiple such selves during a lifetime, if one self is completely replaced by other selves, and whether a person can switch back-and-forth among selves. These questions belong to psychologists and psychiatrists, and perhaps neurobiologists or cognitive scientists. My focus is on the immune self, and what transplantation has to teach us about its nature. Obviously, opinion will differ as to what constitutes the "true" self. Is the immune self a proper representative of the human self, or at least the body's self? This question will not be answered to any great extent here, but it is definitely an interesting one to ask. Suffice it to say for now that the immune self

is but one aspect of the self. One aspect of the self may be all that an honest philosopher may be able to properly understand in a lifetime. Nonetheless, I do believe that some acknowledgement of the other aspects of the self is due, and so these other aspects of the self will now be covered in this introductory section.

The folk concept of the self is primarily a psychological one. This self appears to us as a single, continuous subject ("I") of experiences. This "I" is closely tied to our consciousness, since it is common for all of us to use expressions such as "I" did this, "I" thought of something, or "I" felt that, all of which are highly characteristic of a conscious experience. Thus, the word "I" exists in vocabulary as a semantic tool, and is an indispensable word to describe common, everyday conscious experiences. This conception of the self is sufficient for most people to understand and use. It enables effective one-to-one communication in society and once it is figured out that when I use "I", the word refers to me, but when you use "I" you are referring to yourself, it also allows for unambiguous third-party narratives. Such pronouns are among the first words learned by children. In the context of everyday language, "I" and the self are used synonymously.

Beyond this folk sense of "I" there lies a significantly deeper philosophical tradition. Adi Sankaracharya in the Hindu and Thomas Aquinas in the Christian religious traditions among others, as well as philosophers like Plato, Rene Descartes, and Immanuel Kant were proponents of a mind-body dualism. According to this view there is a distinct, immortal soul linked to, yet always separate from a perishable body. This soul is not definable in material terms because it is independent of physical brain structure. Methods of natural science are unhelpful to analyzing its nature. This soul has continuous experiences that are mediated through the physical body. In the case of Adi Sankaracharya and the Vedanta tradition, the soul is part of a "universal self". These "ego theories" are distinct from the "bundle" theories of the self. Among the proponents of the latter, David Hume wrote that the "I" is nothing but a bundle or collection of different perceptions, rapidly succeeding each other and in continuous flux, with nothing to hold them together (Blackmore 2012).

Similarly, the modern philosopher Daniel Dennett believes that the self is a "center of narrative gravity" (Blackmore 2012) and cannot be considered as an all-or-none phenomenon, but rather should be recognized as a string of narrative assembled to create the illusion of a self. In other words, the self is fictional. Apart from Dennett of course, these ego and bundle conceptions of the self were developed at a time when nothing was known about cell biology or molecular biology, integrative physiology, and biochemistry. The neurocognitive or immunological responses that characterize human existence understandably received no attention from any of these philosophers. As a result, particular philosophical positions can often acquire immunity (pun intended) to subsequent challenge and refutation. Further understanding about the self becomes stalled, and discussion becomes highly theoretical.

Thagard (2014) describes the self as a "multilevel system" consisting of social, individual, neural, and molecular mechanisms. The social self consists of "individuals, families, nations, etc..., and their interactions". The individual self consists of "personal behaviors and the mental representations that people apply to themselves and others" (Thagard 2014). These mental representations are studied according to processes that cognitive science can study, and so can ultimately be linked to neural processes. The neural self consists of neurons and their interconnections. Finally, the molecular self consists of genes and their products, such as neurotransmitters. Ultimately, the self is an integrated system of these four levels of interacting subsystems. Self-concepts operate at these different levels, and exist in an interactive relationship. Rather than describing this schema as a theory of the self, Thagard prefers to call it "a framework for developing specific theories that describe mechanisms that operate within and between levels". Thagard states that this multilevel approach to the self can account for both Kant's unity of the self as well as Hume's diversity of the self. However, he acknowledges that this account does not explain the nature of the self itself.

As a cognitive scientist, Thagard focuses mostly on the neural self, since psychological phenomena of the self will best fit with neural explanations. My general area of focus instead is the molecular self, which as it pertains to cognition, consists of neurotransmitters and their genetic determinants that influence neural firing. Many so-called "phenomena of the self" can in fact be explained by molecular mechanisms. However, my specific focus will be on one component within the molecular self, namely the so-called immune self. Immunology as a branch of modern science seeks to answer the question: why does an immune reaction occur, and how does it happen? The "how", which corresponds to the recognition mechanism of the immune response (or "sensory" arm, to use a neurological correlate) and an effector arm (the "motor" arm), has been worked up extensively over the past century from a scientific point of view, but the "why" still remains an open question. Several theories have been proposed to explain why an immune response is triggered. What is its signal? Is there even a signal at all? Is an immune reaction always present and the immune response, which "succeeds" the immune reaction, present all along and simply an immune reaction whose strength can be clinically detected?

Physicians typically view the immune system as consisting of a group of especially competent specialized cells in our bloodstream and tissues including phagocytes, lymphocytes, killer cells, and natural killer (NK) cells (all are kinds of WBC), which all work together in a coordinated fashion to destroy invading microorganisms (nonself) and thereby preserve host integrity. By analogy, health is preserved by the immune system mechanism as a fortress would be from a military invasion. A transplant is a nonself tissue getting caught in the friendly fire of the immune response because it has been misunderstood by the immune self. Sometimes, the immune system becomes unruly and turns on the body, causing autoimmune disease. The immune system is involuntary: it is not under our conscious control any more than breathing or heartbeat. Disease states result from deficiencies of the immune system, and conversely, a separate set of diseases results from over-activity of the immune system leading to self-antigens being targeted and

destroyed. In this simple model, health is all about preserving a state of balance between immune deficiency and immune excess, between normal immune reactivity and harmful immune responsiveness, and between desirable and undesirable immune responses. The nature of the immune self is not a focus for transplant physicians because it has no pragmatic value.

When a transplant is placed in the body, it must be treated as nonself when the immune system is functioning normally. This simple paradigm functions very well for the purposes of everyday medical practice. It is reinforced by the known effectiveness of empirical practices involving the use of antimicrobial drugs to assist the host's immune system in destroying invading microorganisms, and the use of very sophisticated combinations of chemotherapeutic and immunosuppressive medication to suppress the immune system when it is "inappropriately" acting in excess or is being undesirably targeted to self-antigens.

Infections, cancer, and autoimmune disease are conditions that we can all do without. These conditions are unpleasant to experience at best, and are life-shortening when they are at their worst. We are all aware of someone or the other with one or more among these conditions. On occasion, these conditions may even co-exist. One condition may occur as a result of treatment for the other. A large amount of money is spent on their prevention and/or treatment. New antibiotics and anti-cancer agents are always being evaluated in clinical trials. There is no reasonable hope that any of these conditions will just go away by themselves. Infectious diseases ranging from malaria and tuberculosis, to malignancies such as lung cancer and lymphoma, to autoimmune conditions such as systemic lupus erythematosus and rheumatoid arthritis are all likely here to stay for as long as humankind is around. The immune system is such a central part of life itself that immune-related conditions are invariably part of biological existence.

It would seem that we would do best if there was no situation requiring "provocation' of the immune system, either to be called upon for forceful response in times of dire need to protect us from invasions in a military style of defense from foreign (infection) and internal (cancer) threats, or a situation where it turned upon our own bodies and caused disease by attacking our own cells and tissues. Of course this conjecture is over-simplified and is simply not true, but it is nonetheless a useful conceptualization for clinical practice. A quiet but ever-vigilant immune system is ideal for health, and therefore highly desirable.

However, unlike infections, cancer, and autoimmune disease, transplantation is one clinical situation where we deliberately engage the immune system. Aging of the population due to vastly improved hygiene over the past century coupled with less mortality from war or conflict has enabled the emergence of a new set of diseases of public health importance such as cardiovascular disease and stroke, as well as failure of vital internal organs like the kidneys. Kidney transplantation is far superior to dialysis in terms of replacing lost kidney function. Patients wait for varying periods of time on dialysis before receiving a kidney transplant, and are typically able to return home from the hospital about a week after a transplant. Since dialysis is available as an option for survival, in most situations a kidney transplant is considered to be an elective procedure. Patients with kidney failure are thoroughly screened to ensure they are healthy enough to qualify to receive one, and also to ensure that their quality of life and survival is likely to improve afterwards (for example, patients with active cancer or infection are excluded from transplantation because anti-rejection medication would make these conditions worse). Transplant candidates also undergo an extensive evaluation of their psychosocial health, since the psychological consequences of a transplant can be very significant.

It is reasonable to expect a one-year success rate (i.e., the transplant is still working) exceeding ninety percent and transplant (otherwise called "graft") survival of many years, before the transplant eventually fails (Lamb 2011). When it does fail, the patient then has to

either obtain another kidney transplant if lucky enough, or return to dialysis. Some patients are young enough when their kidneys first fail that they receive more than one transplant during their lifetime. The most common causes for transplant failure are death with graft function (such as from a heart attack, with the transplant still working), or rejection. Patients typically receive three rationally selected anti-rejection medications in combination, in order to keep the immune system's force at arm's length. These medications are continually adjusted keeping in mind that the immune system is lurking in the background, ever ready to exert itself in the event of opportunity or provocation. With transplantation the immune system is engaged at a precisely known point in time, thereby setting the stage for an informative exploration of the nature of the underlying immune self.

In Chapter 2, I discuss how transplantation informs our view of the nature of the immune self. I review the basic components of the immune system, and follow this with an explanation of the two basic types of graft rejection. Next, I provide an in-depth discussion of five major theories of the immune self. I conclude this chapter by providing a uniquely transplant-oriented approach to the nature of the immune self, and by discussing how the five major views of the immune self fit with the real-world clinical experiences of kidney transplantation. In Chapter 3, I explain how the complications of transplantation can help to expand the view I have developed further. I describe what most likely happens when an organ like the kidney is implanted in the alien environment of another human being. Some of the most common and important complications seen after kidney transplantation are viewed through the lens of the immune self rather than the immune system alone. In my summary and conclusion (Chapter 4), all of this information is brought together with a hope to derive a working theory of the immune self, which unlike any previous theory, is based mostly on the transplant experience.

Chapter 2

Transplantation and Engagement of the Immune Self

2.1 The Immune Response

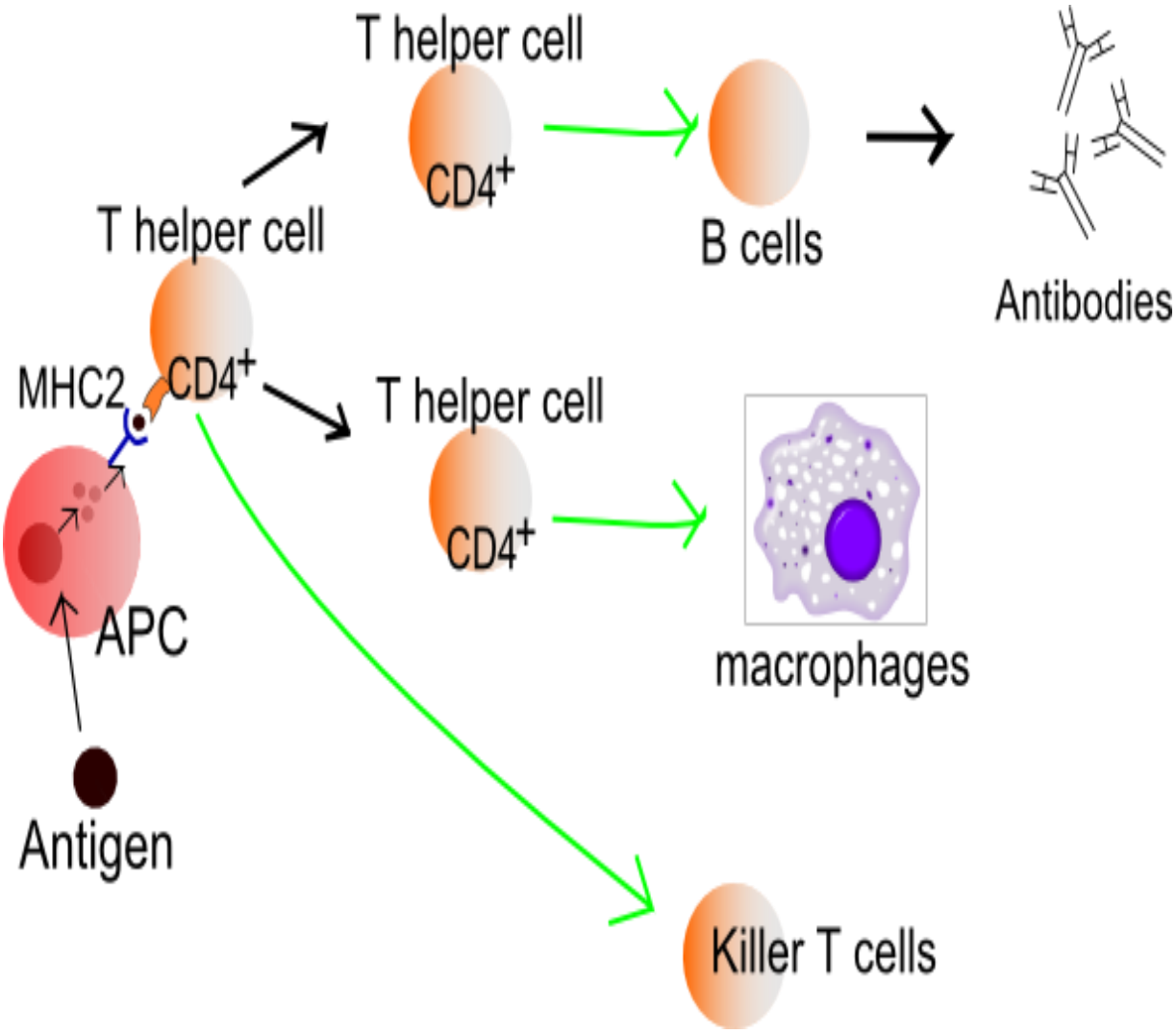
As mentioned in the first chapter, the immune self is what drives the immune response. But what is exactly involved in the immune response? I will provide only a brief summary of the immune response from the viewpoint of transplant clinicians as opposed to immunologists, in order to set out the terminology needed for the philosophical discussion. Understanding the major players in the immune response is needed to understand the immune self, which presumably is at the epicenter of the whole rejection process. The immune response to a graft essentially consists of three phases: recognition of the foreign antigen, activation of antigen-specific lymphocytes, and the effector phase of graft rejection (Danovitch 2005). The basic immune response as it is currently understood is summarized in Figure 1.

The first phase is most important philosophically since we are most concerned with how the graft becomes a recognized target for destruction. The genes that determine graft rejection or acceptance, present in a locus on human chromosome number 6, constitute the major histocompatibility complex (MHC), whose gene products are called antigens. Differences in MHC between the recipient and the graft drive transplant rejection. The MHC encodes for a group of diverse proteins on cell surfaces (human leukocyte antigen, or HLA), and it is the great variation in their structure and function (i.e., their polymorphism) that makes each person unique. Peptides (small pieces of protein) from the donor bind to HLA molecules, and these are then presented to helper T lymphocytes (a subclass of lymphocytes, which are a type of WBC) in the recipient by so-called antigen-presenting cells (APC) that travel from the donor along with the kidney transplant. This presentation occurs through their recognition by the antigen receptors of T lymphocytes (T cell receptors or

TCR). Due to the polymorphism of MHC, many different microbial and other peptides can be bound and presented to T lymphocytes.

Figure 1

The Immune Response



This manner of antigen presentation is called "direct" antigen presentation. Peptides from a transplanted graft get presented to these lymphocytes just like microbial peptides, triggering immune responses. Importantly, peptides from the host ("self" peptides) are also presented in this manner. However, T cells responding to self-antigens are destroyed in the

thymus gland (present in the chest) by “negative” selection, while those specific for “foreign” peptides proliferate by a “positive” selection. In addition to direct presentation, antigens can be presented “indirectly”, which means MHC molecules are shed from the donor cell surface, taken up by APC, processed by them, and their peptides then presented on recipient MHC molecules instead of donor MHC molecules. The distinction between “direct” and “indirect” presentation is important because it is believed that acute rejection depends mostly on the former while chronic rejection depends on the latter (Danovitch 2005).

In the second, or activation phase, more lymphocytes home in on the graft. Lymphocytes get activated through the up-regulation of several biochemical pathways, most important among which is the “calcineurin” pathway. A variety of cytokines, which are chemical products of the cell (like interleukin2 and gamma interferon) are produced and lead to destruction of the graft. In addition to the “first” signal MHC-TCR interaction, there are several “co-stimulatory” pathways (“second” signals), that result from different sites of binding between the APC and the T lymphocyte. In the third, effector phase, there is a proliferation of “cytolytic” T lymphocytes and NK cells. Enzymes like “granzyme” and “perforin” are secreted, which destroy graft cells. Also, “Fas ligand”, a protein, is up-regulated on T lymphocytes, binding to “Fas” on graft cells and initiating apoptosis (commonly known as programmed cell death).

Finally, a completely separate group of lymphocytes called B lymphocytes are transformed into plasma cells (another type of WBC) and secrete a large amount of special proteins called antibodies, which then fix a set of proteins collectively called “complement” to cause “cellular cytotoxicity” (Danovitch 2005).

This model of the immune response, popular among transplant clinicians essentially views the immune self as “fixed”, because it is based on the characteristic traits of the individual donor and recipient, which in turn are defined by their HLA molecules whose

profile is a fixed one, acquired at conception. The transplant is permitted to take place when the HLA profile of both the donor and recipient has been reviewed by an immunologist and they are deemed to be a "compatible" match (i.e., there are no antibodies in the recipient against the donor's HLA). Although a "real-time cross-match", which involves combining the recipient's serum (containing pre-formed antibodies) with the donor's lymphocytes and assessing for lymphocyte destruction, is often performed just before the transplant just to be sure that there is no reactivity, it is nowadays being skipped to save time and perform the transplant as soon as possible. Clinicians have become quite confident in their ability to predict the risk of acute and chronic rejection from such precise pre-transplant information that constitutes what is called a "virtual cross-match". So far, this approach seems to be working well because subsequent real-time cross-matches have been negative.

2.2 Acute and Chronic Rejection

Rejection is the goal of the immune response in the context of transplantation. Broadly speaking, there are two types of rejection: acute and chronic (although the exact terminology for their sub-types is outside our scope). Both can occur independently or they can occur together, but they are very different conditions.

Acute rejection is a common condition after kidney transplantation, typically occurring within the first three to six months after the transplant has been performed. The immune system gets engaged when it senses a "foreign" body part such as a transplant, so lymphocytes and/or proteins called antibodies enter the graft to destroy its cells through a process called inflammation. The urine filters ("glomeruli") and urine passageways ("tubules") get damaged. In more severe cases, even the blood vessels get inflamed. If left untreated, the filters and tubules shut down completely, and get permanently scarred. Blood levels of toxins that the kidney normally removes begin to rise, and eventually the patient experiences kidney failure all over again. Fortunately, most of the time acute

rejection can be “reversed” with more anti-rejection medication if it is detected soon enough. Since anti-rejection medications suppress associated symptoms like pain over the graft or fever, acute rejection often gets detected only through a rise in blood levels of waste products. This lack of symptoms is one reason why regular blood monitoring is performed on all kidney transplant recipients.

Chronic rejection is a term used here for simplicity (the term is actually outdated and inaccurate because it implies that there is always an immune mechanism involved). It was later called chronic allograft nephropathy (Halloran 1999) but it is currently also called IF/TA, or interstitial fibrosis/tubular atrophy (Haas 2014). Chronic rejection usually occurs much later than acute rejection. It is a slow, scarring process that involves the entire kidney, and usually takes place over the course of many years.

Acute rejection can lead to chronic rejection, but a number of other conditions can cause chronic rejection too (Table 1). Often, chronic rejection is simply related to normal or accelerated aging of the kidney (Halloran 1999), and the findings seen on biopsy may be indistinguishable from normal aging, regardless of cause. Lymphocytes and antibodies are sometimes seen in chronic rejection just as in acute rejection, but not always. Eventually the graft fails completely and the patient returns to dialysis or if lucky enough, receives another transplant. In practice, every graft will eventually develop at least some chronic rejection. Anti-rejection medications are not as effective at preventing and treating chronic rejection.

Therefore, the immune system explains real clinical phenomena such as acute rejection and chronic rejection, which are immune responses. However, the mere presence of an immune system is not a sufficient reason for generating an immune response. Acute rejection in particular is directly linked to the actions of various components of the immune system when it is engaged. The rational use of anti-rejection medication prevents acute rejection. Acute rejection points towards the presence of an immune self, because

without the presence of an immune self there is no reason for the immune system to generate an immune response. Acute rejection also proves the existence of an immune nonself because the nonself, which in this case is the graft, is clearly the target of the immune response. Although it is a philosophical term, the immune self is a real entity. Even the course of chronic rejection is determined by the actions of the immune self.

There is a third outcome possible, besides acute rejection and chronic rejection, which is that the patient dies with a functioning graft, and from the same causes as anyone else in the general population. In this situation the transplanted kidney has served the patient well, and it dies along with the patient.

Table 1

Major causes of chronic rejection (chronic allograft nephropathy, interstitial fibrosis/tubular atrophy) (partially adapted from Halloran 1999)

Donor Factors	
Acute	Brain death
	Donor resuscitation
	Organ procurement injury
	Prolonged preservation
	Delayed implantation in recipient
	Reperfusion injury
Chronic	Advanced age
	Hypertension
	Vascular disease
	Genetic
Recipient Factors	
Immune Factors	Anti-HLA antibodies
	Immunosuppressive medication side effects
	Acute rejection
	Infections
	Recipient adherence to medication
Non-Immune Factors	Size mismatch
	Hypertension
	Immunosuppressive medication toxicity
	Recurrence of original kidney disease
	High cholesterol and diabetes

2.3 Theories of the Immune Self

We are now ready to examine the nature of the immune self in detail. In order to do so, we must first review some of the existing theories of the immune self. I have been able to distinguish five of the major theories and describe them next. This list is by no means complete, but it represents the most important theories contrastable against each other in some respect. They can all be examined through the lens of experimental and clinical kidney transplantation. These five theories are: self-nonself theory, danger theory, immune network theory, continuity theory, and liquid self theory (hypothesis).

Definition of Immune Self

We have used the term "immune self" so far without actually defining it. It is probably as difficult to define as an entity like consciousness, so varied definitions exist, although they are really descriptions. Pradeu (2012a) lists five definitions for the immune self: the whole organism, the individual's genome, HLA molecules, peptides presented to T lymphocytes at the time of their selection, and "that which does not trigger an immune response".

Self-Nonself Theory

Most discussion of the immune self brings about an immediate dichotomization of the organism's parts into the so-called "self" and "nonself". The starting point for this theory is trying to explain how tolerance, or selective non-reactivity, to the self is achieved (Pradeu 2006). The "self" was first articulated as a scientific problem by Burnet in 1949 when he wrote, "the failure of antibody production against autologous cells demands the postulation of an active ability of the reticulo-endothelial cells to recognize "self" pattern from "not-self" pattern in organic material taken into their substance" (Tauber 1994). This description is what Tauber (1994) calls the metaphor of the "self". Tolerance is "defined as a specific depression of the immune response induced by previous exposure to the antigen,

and any antigen to which the immune system is exposed during development, up to a certain point, would not be subject to an immune response" (Tauber 1994). More simply put, tolerance is a selective non-reactivity of the immune system to a body part because it is self. Tolerance is the opposite of rejection. Any antigen introduced after the critical time in the organism for defining the immune self has elapsed is destroyed by the immune response.

However, the concept of "foreignness" as the instigator of the immune response had been espoused many years before, independently by Metchnikoff and Ehrlich in the early 20th century, when they promoted the phagocyte and antibodies as the principal effectors respectively (Pradeu 2012a). Experiments by Owen and Medawar in the mid-20th century were able to demonstrate that the capacity for differentiating between self and nonself is an acquired one. For example, a second transplant is typically more quickly rejected than the first (Pradeu 2006). Tolerance to foreign antigens can only be induced in embryonic life, but not later. Burnet felt that the self-nonself distinction was a process of Darwinian selection of antibody-producing cells at the cellular level (Burnet 1991). Cells implementing an immune reactive response are selected on the basis of HLA antibodies that they carry on the surface of their cells.

Immune reactions occur as a result of the interaction between the self and nonself particles. The immune self is thus of genetic origin (an "immunogenetic" self), appearing early in evolution, and whose teleological purpose is to protect against pathogens, by identifying them as nonself particles. Immunology by extension is the "science of the defense against the nonself, while respecting the self" (Pradeu 2012a). Others (Zinkernagel 1978) showed that T lymphocytes expressing self-antigens on their surface were eliminated in the thymus gland, thus ensuring "protection" of the self. Another supportive piece of evidence for the self-nonself theory was the demonstration of somatic recombination, in which a small number of genes is sufficient to synthesize a huge number of diverse antibodies (Tonegawa 1983). Further extensions of this theory include the "peptiditic self",

with the self being defined by the peptides presented, not genes, at some point of "immune maturity" (Claverie 1986).

The self as it is understood here is still quite broad in scope. It could include everything encoded by the genome, any tissue accessible to lymphocytes, the set of peptides associated with the MHC, specialized cells such as APC or soluble molecules of B lymphocytes, or a set of body proteins existing above a certain concentration (Pradeu 2006). The nonself could include invading foreign microorganisms, or anything else that the organism deems undesirable, including host tissue that gets assigned a "foreign" label. In modern times, the nonself exposure could be a transplanted organ, as we have noted.

This self-nonself paradigm has no doubt been a useful one for immunologists and transplant clinicians over the years. The immune system thereby defines the "individuality" and protects the unified individual identity over time through the "maintenance of integrity". Burnet's theory (Mackay 1957) provides us with a very useful starting point for our exploration of the immune self because it seems intuitive, and seems to be able to provide us with a working ontology of self-preservation.

However, the validity of the self-nonself distinction has been repeatedly questioned, with the result that there are now multiple alternative theories about how the organism maintains its integrity in the face of the innumerable challenges it faces in the course of the continuous interactions with its environment. Even the very usefulness of the self-nonself distinction has been questioned (Pradeu 2012a).

The main criticism of the self-nonself theory is that the body contains a large number of nonself entities such as microorganisms (and even large parasites) that are being perfectly tolerated. Also, not every immune reaction generated from a self-nonself distinction leads to an immune response. Autoimmunity is, in fact, a normal process that defies the need for destroying nonself. Some cells like NK cells do not respond to nonself, but rather to the absence of self on cells. Despite Zinkernagel's findings, lymphocytes

strongly reacting to self-antigens are still capable of surviving by escaping destruction in the thymus. Moreover, the thymus selects for survival not those T lymphocytes that do not react to self-antigens, but those that react weakly to them. This reactivity is also true for B lymphocytes, and these interactions are in fact necessary to their survival. Even regulatory T cells, whose role is to inhibit the activity of other lymphocytes and thereby curb the immune response, react with self cells (Pradeu 2012a). Autoimmune disease is the result of normal autoreactivity evolving to become an immune response.

The self-nonself theory has led to some offshoots. The clonal selection theory is one such offshoot that has been attributed to both Jerne (1994) and Burnet himself, before he began to favor a genetic model (Pradeu 2012a). According to the clonal selection theory, the antigen "selects" from among naturally circulating antibodies of random specificities those with which it has a natural affinity, combines with them, and then delivers them to cells that are capable of producing specialized proteins called "immunoglobulin". When the antigen-antibody complex enters such cells, the antigen is released and induces the synthesis of identical "specific antibodies". This process is refined and gets strengthened with continued antigen exposure, but eventually subsides to an equilibrium state once antigen exposure ceases. Antibodies that do not attach to cells that are capable of synthesizing immunoglobulin are destroyed. In this theory, there is no actual mention of the self because it was proposed as a model for the immune response only. Nonetheless, it accounts for "learning" as an explanation for the observation that with repeated exposure to the antigen the immune response becomes more specific and forceful, with the "right" kinds of cells being produced in larger numbers. The clonal selection theory also accounts for tolerance, in which there is no immune response, because clones of cells that recognize the self are effectively eliminated during embryonic life.

Danger Theory

The second important theory of the self we will discuss is a widely known, easily understandable, and therefore appealing theory (Matzinger 1994). According to the danger theory, every immune response is due not to the presence of nonself, but to a tissue's "emission of danger signals". The immune system reacts to such signals that emanate from damaged tissues or cells, and these could be of either endogenous (host) or exogenous (foreign) origin. This danger is recognized by APC (cells we met earlier). The distinction between self and nonself is actually not relevant to immunology, but rather, the important distinction really lies between what is dangerous and not-dangerous. The ultimate signal is therefore of endogenous tissue origin. Thus, control for the immune response rests not with the immune system, but with the target tissues themselves. Healthy tissues induce tolerance, but when tissues are distressed they stimulate immunity. The type of tissue being affected may also determine the type of effector cells involved (Matzinger 2002). Matzinger uses the example of a type of immune response called delayed type hypersensitivity as being very useful for the skin, but very harmful for the gut, a site where secretion of immunoglobulin is the more appropriate response. The type of response that occurs is based on the release of specific local tissue signals. Thus, immunity is controlled by "an internal conversation between tissues and the cells of the immune system" (Matzinger 2002). It is claimed that this will explain states of tolerance such as pregnancy, as well as autoimmunity and individual differences in transplant acceptance. This theory fundamentally differs from the self-nonself theory by not placing the emphasis on biochemical specificity as a determinant of the immune response.

The danger theory also blends in with the so-called "damage" theory. Damage represents one step further down in the pathway from a mere "danger" to the tissue. APC are activated by alarm signals from cells injured as a result of microbes, toxins, or even mechanical disruption. These alarm signals may be constitutively present in the cell, or their production may be induced in response to a stimulus; they may be released as a

result of cell lysis (destruction) or they may be actively secreted by the cell. These signals do not get emitted from healthy cells or cells undergoing normal death (apoptosis). Thus, mere "self-ness" is no guarantee of tolerance. The danger model thus explains "what the immune system does right, and what it gets wrong". The danger model provides an explanation for the "dangerous self and the harmless foreign" (Matzinger 2002).

The danger theory has been extensively criticized as well. The danger theory is a good model for adaptive immunity but does not provide an adequate explanation for innate immunity. Innate immunity does not require an antigenic stimulus in the same way as adaptive immunity requires one. The actual damage signals have not been defined on a molecular basis (Pradeu 2012a), making the danger theory vague. Furthermore, it is known that antigens can even provoke an immune response without there being any preceding damage (Vance 2000). Moreover, inflammatory signals alone do not suffice to activate APC. It has been shown that specific interaction with the antigen is needed (Joffre 2009). Most tissue damage actually occurs due to the action of cells from the immune system, with very little if any damage preceding it. Thus, there is confusion between the reason for the immune response and its effect (Pradeu 2012a).

If the danger theory is correct, tissue damage that occurs as a result of the transplant operation ("the surgeon's hand") should then be the cause of an immune response rather than any self-nonself difference, but in that case, auto-transplants (where one body part is transposed to another) should also result in an immune response. This obviously does not happen (Pradeu 2012b). If immunity truly depends on endogenous signals, then it is difficult to explain how many microbes are effectively eliminated before they have ever had a chance to cause any damage. Finally, unlike what Matzinger believed, tumors do elicit immune responses and many incipient tumors are eliminated in this manner (Pradeu 2012b).

Immune Network Theory

This theory is credited to Jerne (Jerne 1974). The immune reaction does not consist of the actions of a small group of highly specialized cells, but rather involves multiple interactions among many different kinds of cells (Pradeu 2012a). Since most of the cellular components of the network are endogenous, the immune system is directed primarily inwards, towards the self. Self-organization is the guiding principle for this network. Antibodies are produced initially when there is no nonself stimulus and serve to regulate the organism's structure. However, any antibody, since it is a protein, can itself become an antigen at any time, and result in the production of so called "anti-idiotypic" antibodies. The immune response begins in this way, rather than by the recognition of a foreign antigen. Thus, the immune system only reacts to its own disturbances. Antibodies generated as a reaction to foreign antigen are created in the image of self antigen. This theory has been very helpful to the study of autoimmune disease. Jerne also draws an analogy to the nervous system. He writes (Jerne 1974) that the "activation-inhibition processes in the immune system are very similar to those seen in the nervous system". He also claims that the interactions among immune cells amounts to a "language".

An extension of the immune network theory is that of "autopoiesis" which essentially denies the existence of any nonself. Foreign substances simply interfere with the organism's own normal processes and the networks among them. All immune reactions are in essence autoimmune, and external interference leads to "cross reactions" (Vaz 1978).

As expected, the immune network theory is not immune to criticism. It has been accused of vagueness by Jerne himself (Jerne 1974). Moreover, it is now well-known that autoimmunity depends more on T lymphocytes than antibodies. The use of metaphors involving the nervous system and mind can be misleading by attributing cognitive abilities to immune cells, and can stifle further research into the nature of the immune self (Pradeu 2012a).

Continuity Theory

This fourth theory belongs to Pradeu (2006), who had extensively criticized the preceding three theories. Pradeu writes, "the triggering of an immune response is due to a strong modification of the antigenic patterns (ligands) with which the organism's immune receptors interact, which is to say a sudden appearance of patterns in the organism that differ strongly from those with which the immune system is continuously interacting" (Pradeu 2012a). The distinction being made here is between "long-term presence versus sudden appearance" of an antigen, as opposed to "never met versus already met". There is a transition from a normal, baseline autoreactive state to a state characterized by a stronger level of reactivity. The continuity theory does not attempt to explain why immune responses occur but focuses instead on claiming conditions under which a "normal, constant autoreactivity" gets upregulated to become an immune response. The emphasis is on the strength of the difference between the antigen and the normal molecular patterns that the immune system encounters. The "continuity" in the continuity theory refers to points in time after immune competence is acquired. Immune competence may develop over several stages in the organism, and in humans this rapidly develops in the few weeks following birth. Immune tolerance thus comes before immune responsiveness. The neonate must "tolerate" its invasion by colonizing microbes, which are essential to its future health. The transition is thus not from immune deficiency to immune competence, but from a state of more tolerance to less tolerance. The antigens that initiate the immune response may be either endogenous or exogenous. Autoimmunity is an important process without which the organism cannot survive. In early human life, most immunity is innate (Adkins 2004).

Factors that determine the immune response include the quantity of antigen, the speed of its appearance, degree of molecular difference, the regularity with which the antigen is presented, and the specific site in the body where the interaction takes place (Pradeu 2012a). The quantity of antigen must be sufficiently large to be recognized by APC. If the quantity is too small there is a state of "active immune tolerance" (Pradeu 2012a) and

if too large, the immune system may be paralyzed. If the antigen is introduced suddenly, then an immune response is more likely. The degree of molecular difference must be sufficient to elicit an immune response. If the molecules are very similar, then an immune response may not be generated. If antigens are introduced at regular intervals, again an immune response is more likely to take place. Finally, the site in the body where the antigen-host cell interaction takes place may be important, since a reaction may take place if the interaction occurs at one site but not another.

Pradeu claims that the continuity theory is compatible with the well-known finding of immune surveillance (Burnet 1957), in which NK cells are constantly looking for cells that do not contain self-identification, so that they may destroy them. Rather than detecting nonself as such, the immune system is recognizing "strong molecular change" to differentiate the nonself from self. Immune tolerance is more easily explained by the continuity theory in this way than by the self-nonself theory, because the state of tolerance is constantly changing. A state of tolerance is achieved if the antigen does not meet minimal conditions for activating the immune system, mutates, actively inhibits the effector response, or actually induces continuity.

Continuity explains the well-known tolerance to the fetus as well as the phenomenon of "chimerism". Chimerism is a state wherein cells of fetal origin can be detected in the mother many years after a pregnancy has been completed. These cells exist in perfect harmony with the mother's cells and do not generate any immune response because they do not meet the condition of strong molecular change required for activation of the immune system, according to Pradeu's criteria.

Pradeu also claims that the continuity theory provides a useful framework to guide future experiments in immunology. Pradeu does not specify exactly what he means by "change" or "difference" at the molecular level, but leaves it to experimental immunologists to determine such thresholds for eliciting immune responses.

Liquid Self Theory (Hypothesis)

Our fifth theory, referred to as the liquid self hypothesis by its authors, is the most recent theory to be proposed and is perhaps the most holistic in terms of its framework. The "liquid self" (Grignolio 2014) denotes that molecules and cells, as well as associated microorganisms such as bacteria and viruses can switch back and forth between a "self" and "non-self" status. This self or nonself status depends on their antigenic potential at any given point in time. It is proposed as an extension of the danger/damage theory, but distinguishes itself from the danger/damage theory by claiming that there is a "full integration of the immune response mechanisms into the host body's ecosystems". The body goes through numerous changes as part of the evolution process, and this in turn "reshapes" the antigenicity of various potential antigens so that they go back-and-forth between self and nonself status. Environmental factors such as nutritional state, lifestyle, and geography all have an influence on antigenicity. Thus, besides molecules like antigens and antibodies that determine immune reactions, time and space also play a role.

As an example, in the right environment, gut flora become part of the self. Aging alone can result in self evolving into nonself. The liquid self hypothesis differs from other theories by not being "suspended in a limbo without time and space" (Grignolio 2014). Metaphorically, the liquid is compared by analogy to the "rapidly changing values of contemporary Western societies" (Grignolio 2014). It views itself as an improvement upon the danger theory by taking factors not traditionally associated with the host into account. The liquid self hypothesis has been described by its authors as more "procedural', i.e., time and space-related, rather than "materialistic" (cellular or molecular).

According to the liquid self hypothesis, the immune self is a continuous entity and cannot be strictly dichotomized into a self and a nonself. The immune self is "dynamic" and is a "process". The immune self varies based on the immune reactivity of the host and the environment, which themselves are undergoing continuous change. By this view, the

self is a continuum of states based on the "quantitative, qualitative, and temporal aspects of the immunological stimuli that each of us is exposed to in our lifelong history" (Grignolio 2014). The temporal aspect in particular is exemplified by the unique antigenic exposures of birth (Huurre 2008) and old age (Franceschi 2000). It can explain the inflammation associated with aging ("inflammaging") and the spread of inflammation to bystander cells ("paraflammation"). The liquid self hypothesis explains why food particles in the gut are not antigenic, because they are not considered as nonself by the immune system in that location. It has also been proposed as an improvement upon the continuity theory because it takes the environment into account as an important factor beyond the genetic profile of change (Baedke 2013). The liquid self hypothesis introduces a new term, "immunological biography", to denote the temporal and environmental dimensions of the immune response in the body.

The liquid self hypothesis also emphasizes that the particular genome which exists is the result of selective evolutionary forces at a population level. Microbial infections exert selective pressure over a single lifetime as well as in populations. This hypothesis is partly compatible with the continuity theory, since immune reactions can be triggered by sudden changes in amount of antigen exposure, such as from an invading microbe. The absence of a known microbe associated with a genomic change simply means that the microbe has not been identified. One of the main reasons for the wide MHC diversity is to enable at least some members of a population to survive microbial onslaught. It is the result of "pathogen-driven selective pressure" (Prugnolle 2005), so that there is an equilibrium among "fighting invaders, tolerance of self, and innocuous antigens" (Sironi 2010). According to the hygiene hypothesis (Strachan 1989), we now have to deal with fewer microbes as a result of improved public and personal hygiene, and so autoimmune reactions become favored (Sakaguchi 2009). Some pathogens like worms in the intestine are ignored by the host (Rook 2009), as can be seen in African populations. However, this is not seen among European and Asian groups (Pennington 2009).

One can compare the liquid hypothesis of the immune self to the malleability of the psychological self (Markus 1986). According to the malleable self-concept, the malleable property of the self is derived from continuous social interaction. Each piece of challenging information thus presented in a social environment results in the activation of different substructures in the working self-concept. The individual "constructs a working self-concept that integrates the core self-conceptions with those elicited by the immediate context" (Markus 1986). The working self-concept is thus "quite situation dependent" (Markus 1986). When people are presented with information about themselves that "threatens" their core self-perception, they strive to reaffirm that core self-perception. This view of the psychological self is remarkably similar to the liquid hypothesis of the immune self. In both cases the self is highly dependent on the environment.

The meaning of "malleable" and "liquid" in this context is essentially similar. Malleability and liquidity (as the case may be) are both built upon a core of stability, consisting of self-confirmation and molecular signatures respectively. In the case of the liquid self, rejection is the self-affirming response to a disruption in the immune self. A stimulus may be assigned either self or nonself status depending on the context of the interaction. It is very interesting to speculate that this paradigm of environmental dependence of the immune self can be extended to other aspects of the self as well.

2.4 The Transplant Perspective of the Immune Self

An Introduction to the Immune Self in Transplantation

Having outlined the five major theories of the immune self, I will now attempt to reconcile these theories with the clinical experience of kidney transplantation in order to derive a transplant-focused theory of the immune self. I start with the supposition that none of the five theories is entirely correct, but also that there is explanatory value to transplantation in all of them. It is quite possible that a new theory could emerge from a synthesis of the existing theories that can more adequately explain real world observations which have already been subject to scientific explanation, yet still lack philosophical grounding. An example of such a question would be: why is there such an entity as acute rejection? It is equally possible that several clinical observations that cannot be explained on the basis of scientific experiments may be better understood by utilizing a more theoretical approach, so that future scientific experiments of explanatory mechanism will be more likely successful. An example of this type of question would be: all things being equal, why do some transplant recipients develop acute rejection and other do not? Seeking answers to such questions is one of the roles for the philosophy of science and medicine, and I am seeking here to apply this to the philosophy of transplantation.

A starting point for composing an immune theory of the self is to seek the nature of the self by first analyzing the metaphor. In his book "The Immune Self: Theory or Metaphor", Tauber (Tauber 1994) argues that understanding the "self" metaphor requires a deeper understanding of "immunology's governing agenda". Metaphors and theory share an underlying structure, and thus "their meanings are reciprocal and intimately linked". Tauber describes the early research of Metchnikoff with the voracious phagocyte, which Metchnikoff believed was the enforcer of the body's self-nonself distinction. The phagocyte is quite capable of engulfing and destroying bacteria as well as dead or dying endogenous cellular elements. Phagocytes can be seen in the graft on biopsy. Tauber also references the

philosophical work of Nietzsche, who discusses the self on the basis of its continuing interactions with its environment and the struggle itself being the goal of life. He takes it one step further however, by describing the whole body itself at a given point in time as being the ever-changing end-product of an ongoing struggle for survival not only with the environment, but a struggle amongst various constituent body parts. The contents of the body that we recognize today emerged as a result of a struggle with other body parts for survival. An immunological response is thus not only a distinction of self from nonself, but also, and perhaps more profoundly, the self's own method of evolution. It is, in a manner of speaking, affirmation of the "primacy of the will" through an outwardly directed dynamic process (Tauber 1994). Perhaps the transplant is just one more organ that must now join this struggle among the organs. Although these five theories each provide us with a purpose for the self, just like Nietzsche did (Tauber 1994), they do not give us enough information about the nature of the immune self. The immune self metaphor is compatible with all of them.

Tauber makes several references to transplantation in his book. Transplantation is an obvious challenge to the sense of an integrated self. He refers to tumor transplantation in inbred mice strains and how tumor rejection is an immune reaction directed against alloantigens. Elsewhere, he describes the MHC as the molecular "signature" of the organism. However, Tauber does not develop ideas specifically related to transplantation, such as an immune self contextualized to transplantation.

Of course, we now know that it is the lymphocyte, not Metchnikoff's phagocyte, which is the principal effector of the acute rejection process. Phagocytes are seen more commonly in bacterial kidney infections. New scientific discoveries in immunology made over the past century have confirmed or refuted many theories. Tauber concludes, "that immunology has been using a metaphor (that) yields significant insights into its workings...To move into the strata where metaphors abound and govern the discourse is not only to acknowledge our ignorance, but to exhalt that a new vision is beckoning". The

metaphor of the self is employed generously in my thesis. Transplantation cannot escape the metaphor because it cannot provide a description of the governing agenda, although it may more modestly help to explain some of the underlying mechanisms and the reasons behind them.

What is Unique about the Transplant Situation?

Even if all five theories have something to contribute to a delineation of the immune self in transplantation, narrowing down the field of theories may help to understand the immune self better. My starting point is the supposition that the immune self is unique to the individual, and the immune self changes as a result of the transplant. Tolerance, towards which transplantation science strives, represents successful incorporation of new components into the self, thereby creating a new, stable self. In some cases there is rejection, in which case the immune self acts in a hostile manner by initiating an immune response. In other cases there is complete acceptance of the organ, in which case the immune self is being accommodating of the new antigens. Burnet's definition of the self (the metaphor) is a negative definition. A positive definition of the self, however, would be much more appealing.

In the case of transplantation, there are several specific additional factors that need be taken into account when designing a transplant-centric theory of the immune self. The self is being suddenly redefined by the incorporation of a new organ. As a result, the immune system is provoked, and an immune response occurs. This immune response, rather than the immune self, is the target of anti-rejection medication therapy. The immune system is as healthy as it is ever going to be at the time of transplantation. True tolerance, which is the absence of destructive immune responses to transplant tissue without ongoing anti-rejection therapy, does not seem to be an attainable goal in the foreseeable future although varying degrees of success have been reported (Kawai 2014). With very few exceptions, anti-rejection medication is always required for the life of the transplant, and

so it is almost impossible to ethically perform human studies of transplantation and its associated immune responses without there being molecules of immunosuppressive drugs present in the environment being studied. This limitation might be overcome through animal experiments or in-vitro studies, but there can never be direct observation of what is occurring in the patient. Even a transplant kidney biopsy, widely considered to be the "gold standard" of transplant assessment, is only a frozen image of a tiny piece of the kidney taken at some point in the past, separated from the rest of the kidney. What is seen in the biopsy is a representation of the kidney's status with the immune self, as viewed by the transplant clinician. That the immune response is deliberately suppressed with medication to enable successful transplant organ function needs to be reconciled with the fact that a transplant invariably carries along with it at least some side effects, both attributable to and not attributable to immunosuppression. This need for balance is the case with virtually all patients, since all will experience at least one medication-related side effect. These side effects are the consequence of the blunt targeting of the immune response without considering the nature of the underlying immune self which has been redefined by the transplant.

Since there is a clear target for the immune response in the case of transplantation, which is the graft, there is a nonself (or immune nonself). This nonself includes everything that is the target of the immune response. There will be body parts that are in molecular continuity with the rest of the body but are considered nonself by the immune system, such as cancer and invading microorganisms. On the contrary, there will be parts not in molecular continuity (such as food and helpful gut flora) that are considered self. Therefore, the immune nonself is not definable based on physical body integrity alone.

In sum therefore, the immune response to a transplant, with all the above caveats and more, could still be nothing but the result of a further evolution in the self, in the face of one or more additional challenges. Moreover, the so-called immunological adverse effects of transplantation, that range from acute rejection on one hand to post-transplant

infection and cancer on the other, are simply adjustment processes that occur during the attempt by the self to re-establish itself in the face of this challenge.

The Self-Nonself Theory Works

The self-nonself theory is an excellent place to start developing a transplant-centric theory of the immune self. Clinical transplantation is still practiced according to the self-nonself theory. Donors are still selected for recipients on the basis of their HLA profile, and such selection is likely to be the case indefinitely. Long-term kidney transplant survival is best when the donor and recipient are identical in their HLA molecules. A positive cross-match due to non-HLA antibody is not considered to be as clinically significant, but the presence of preformed donor-specific anti-HLA antibodies is very detrimental to the graft. Conversely, if the cross-match against one's own cells is positive, then a positive cross-match against a donor is not considered as significant. This concept of the self is easily understood from a medical perspective, and information regarding the self's signature as expressed by the HLA system is regularly used to guide decision making in clinical transplantation medicine. This includes donor selection and approval, as well as managing the selective type (for example, whether to use immunoglobulin as therapy) and intensity of immunosuppression after the transplant. Even if there is some fluctuation in the amount of specific anti-HLA antibodies over time, it is the most recent amount, or "titer" of antibody that is given the most importance. The self-nonself distinction is a system that clearly works.

It is well established that heart transplants can be performed in very young infants across blood type barriers without the need for additional immunosuppression. This window of opportunity is lost after a certain age is past. There is ample evidence that immune tolerance exists in this situation (West 2011). This important clinical advance in transplantation is fully compatible with the self-nonself theory, in that transplants can be

freely performed across many different types of barriers until immunological maturity is reached, but not after this.

One practice in clinical transplantation is the use of "protocol biopsies", according to which biopsies of the kidney transplant are taken in order to look for acute rejection even though kidney transplant function is apparently normal. Protocol biopsies are typically performed at fixed points in time after the transplant, for example six months or twelve months later. Initially, studies found that there were lymphocytes present within the graft, cells that clearly belonged to the recipient and not the donor. Acute rejection was then presumed and the patients were treated with enhanced anti-rejection medication. It was found that transplant function improved as a result. Numerous other types of "immune monitoring" can also be performed nowadays (Ho 2012).

The philosophical questions regarding protocol biopsies are: what were the lymphocytes, found in the early studies with protocol biopsies (Rush 1999), doing in the graft in the first place? Were they present to just "survey" the graft and make sure that it was not harmful to the self? Were these lymphocytes essential to the graft's accommodation and consequent survival? Or had the immune self already decided that the graft was a threat and needed to be destroyed, sending in these lymphocytes as part of an immune response? Cell markers to distinguish type of lymphocyte present seem to indicate that they seek to cause harm, but remember the biopsy is only a snapshot of the past and the particular graft is still working well in the present. Perhaps nothing would have happened and the lymphocytes would have gone away by themselves.

Finally, a lower amount of immunosuppression (for example, using one or two medications instead of three) can be used in HLA-identical situations. Knowing that graft life should be maximized, extra "points" are given to waitlisted potential transplant recipients who have a very large number of different anti-HLA antibodies in their blood. In Canada there is a national kidney sharing system in place, in order to be able to perform a

transplant in such highly sensitized patients for whom a kidney may never be found otherwise. This means that if a compatible donor is found anywhere in the country, against whom the potential recipient does not have specific anti-HLA antibodies, then their kidney gets allocated to that recipient. It might represent their only chance of ever receiving a kidney. It is generally agreed that grafts implanted in recipients who do not have preformed antibodies against their donor do better over both the short- and long-term.

In sum, the self-nonself theory works across many aspects of clinical transplantation. When things are going well, it means the self-nonself distinction has been properly understood and respected during decision-making.

Limitations of the Self-Nonself and Other Theories in Transplantation

Nonetheless, there are still some limitations to the clinical use of the self-nonself theory. Acute rejection can still occur with very close matching, there is "cross-reactivity" across HLA epitopes, and anti-HLA antibody responses can be mounted against certain non-self HLA antigens, but not others. Furthermore, a common post-transplant event is that the graft can become very unwell yet nothing at all is seen on the biopsy. There is poor correlation between what is seen on the biopsy and what happens clinically. Such cases are puzzling to solve, but not uncommon. Sometimes a second graft works much better and lasts much longer than the first, going against the phenomenon of immunological "learning". The correlation between the WBC count and risk for rejection is not that good. All of these clinical observations lead us to the crux of the self-nonself theory's validity. The self-nonself theory is not very helpful for predicting what happens after the transplant. After a transplant, the immune response is being targeted without explicit knowledge of the status of the immune self.

Rejection of course favors the self-nonself theory. If the grafts are well-matched by HLA antigens and rejection still occurs due to our current limitations in technology, whatever they may be, it could still favor the self-nonself theory. Can the danger theory

help us here? Fortunately, the kidney is not immunogenic in the same way as a bone marrow transplant. The kidney does not cause graft-versus-host disease (GVHD), which can be fatal. However, in the case of rejection the immune self would have failed to recognize that the kidney graft here is an ally and not an enemy. The graft is not meant to be a danger. Damaged kidney grafts of course are not uncommon after transplantation, because of injury that can occur in the process of death in the donor, during transportation, and during implantation if it does not receive enough oxygen or nutrition (see Table 1). Although there is a slight increase in acute rejection with a damaged graft, overall it is not that common or necessarily more severe. Graft rejection is not a fatal event. Some data indicate that patient mortality may be increased with graft damage independent of its recovery of function (Narayanan 2010). If this finding proves to be correct, it might indicate that the danger and damage have spread to the rest of the body. However, it is not necessary that the immune response be involved in this case.

If one considers the whole body as an immune network, it would also favor the danger theory. However, there are no transplant-specific data to support this claim, and in most cases of rejection there are no systemic symptoms. A transplant does not trigger any autoimmune disease. The patient typically feels perfectly fine, other than perhaps experiencing some symptoms related to kidney failure all over again. This basic clinical observation goes against both the danger theory and the immune network theory. The immune network theory, which is essentially a model of communication among cells rather than the actions of specific cells, does not seem to be that helpful to transplantation because it has better explanatory value for autoimmunity, and in the case of kidney transplants autoimmunity is not a significant problem. HLA autoantibodies are not routinely produced. They are seen mostly in the context of autoimmune disease, and do not have important consequences for transplantation. “Cross reactions” from non-significant antigens seen during the cross-matching process before transplantation are usually ignored.

With regards to the continuity theory, there is nothing more sudden in terms of an immune challenge than the implantation of a graft. Nonself HLA molecules are suddenly introduced, ready for surveillance and even interrogation by the recipient's immune system. There is discontinuity in this context. However, there is little correlation between the amount of discontinuity and the propensity to rejection, at least based on the degree of HLA matching. It does not matter whether there are 5/6 antigens matched, or 4/6, or even 0/6. The effect on long-term graft survival is also trivial. Modern anti-rejection drugs which effectively target the immune response have rendered any effects of HLA matching largely irrelevant. In the case of a heart or lung transplant where the transplant must be done to save the patient's life, the HLA profile may not even be checked. The graft is physically very large, full of unfamiliar antigens, but it never "paralyzes" the immune system, as the continuity theory predicts might happen. There is also no correlation between the anatomical location of the kidney and the propensity to rejection. In fact, often the left kidney is transplanted on to the right side. Pradeu admits that the continuity theory does not attempt to explain why immune reactions occur, only to explain why normal autoreactivity gets upregulated to an immune response. By definition, there is no "auto" reactivity to a graft. If it is autoreactivity that is getting upregulated, it should not explain the graft's "change in status".

A graft typically lasts many years in the body, even after it has failed, because it is often left in place to avoid a second surgery to remove the graft. However, it can sometimes be acutely rejected many years after it has failed, which then necessitates its removal. Rejection happens because drugs are tapered, not because of any discontinuity in the HLA profile. Anti-rejection medications are targeted to affect downstream cellular pathways, not HLA molecule expression. Therefore, the continuity therapy is not very helpful for our purposes.

The Liquid Self Hypothesis is Most Compatible with Transplantation

As I have emphasized, the clinical course of transplant patients is highly variable, despite all our knowledge of HLA molecules and the immune reaction mechanism. Although transplant professionals strive to achieve maximum transplant success, pragmatically summarized as a state of optimum health and prolonged graft survival, this does not always happen. Despite intensive screening, highly sophisticated surgery, and dedicated post-transplant care, some patients do very well, some less well, and others do very poorly. Clinical acumen is just one tool at the disposal of clinicians, and is sometimes inaccurate in predicting how a given patient performs. Despite the many tests that have been specifically developed to assess and monitor the immune system, the immune performance of the graft cannot always be predicted. Some patients will not have a rejection despite pre-transplant testing that predicts a high likelihood for such a complication, while others develop severe rejection which none of the tests predicted. One patient will develop complicating conditions x_1, x_2, and x_3; another will develop x_4, x_5, and x_6; while yet another will develop x_2, x_4, x_7, and x_8. The sequence of development of these complications is also variable. Such variability can be adequately explained only by a liquid self.

It is becoming increasingly clear that the "body" also includes the environment. The lumen or cavity of the gastrointestinal tract from mouth to anal canal, strictly speaking, is not part of the human body. Large amounts of water can be retained in the stomach and later get absorbed further down the gastrointestinal tract. Urinary tract infections occur because of a trans-migration of bacterial species from one body compartment to another, such as from the large intestine to the urinary bladder. Urinary tract infections are a common occurrence in transplant patients and may be precipitated by activities such as poor oral fluid intake or sexual intercourse. Further spread to other compartments can occur through the bloodstream as a result of the immunosuppressed state. With a kidney transplant, the anatomy of the urinary tract is altered, leading to a mechanical reason for recurrent infections. However, the organism may not be the same every time. The identity

of the offending organism is assumed when an antibiotic is prescribed initially, but the antibiotic may need to be changed when the results of the urine culture are obtained, because the sensitivity of the organism to antibiotics may also change. Viruses like cytomegalovirus (CMV) and herpes simplex virus persist in a dormant state in the body for many years, even without a transplant being performed.

Therefore, the body is host cells plus many other types of cells. The immune self acknowledges this and deals with this, by attempting to establish equilibrium in the context of its internal and external environment. This equilibrium is no doubt fragile and affected by aging, since older transplant patients are more likely to get opportunistic infections and cancer while younger patients are more likely to develop acute rejection. Their anti-rejection medication strategy is designed accordingly. Undesirable outcomes still happen because we do not have an "immune meter" which can measure the extent to which an individual patient has been immunosuppressed. All we have to guide us is a vague "propensity" to infection or cancer (if a patient gets these recurrently), as well as anti-rejection drug type, dose, or in some instances, measured blood level. A lot depends on the immune self's ability to reestablish this equilibrium on its own, while clinicians in the meantime exclusively focus on the graft's function, and the immune response to the graft.

Most infectious disease and cancer that occur in the recipient are of endogenous origin. Viruses in particular stay in the body lifelong once they have been acquired. Examples include CMV and BK virus (BKV, named after a patient), as well as the urinary tract bacteria described above, which can all be long-term residents of the body. If an infection is transmitted from the donor along with the transplant, the effect can be especially hazardous. For example, CMV mismatch (donor positive for antibody against CMV, recipient negative) is a risk factor for serious CMV disease, while Epstein Barr virus (EBV) mismatch (donor positive for antibody against EBV, recipient negative) can actually lead to lymphoma. If the infection is directly acquired from the environment however, the effect is not likely to be as severe. Cancer can also be transmitted along with the graft.

Under immunosuppression it grows as it probably would have in the donor, and metastasizes in the same way, perhaps more quickly. Some de-novo cancers are especially common in transplant recipients. Skin cancers are especially common as a result of excessive exposure to ultraviolet light from the sun, as in the case of transplant recipients in Australia, while some cancers which are extremely rare like Kaposi's sarcoma (seen in human immunodeficiency virus infection) become much more common than before, even though they still remain relatively rare. Sometimes cancers resolve with minimal intervention. These observations result from the immune responsiveness being altered in various ways. The mechanisms for these diseases still need to be fully worked out, but there is clearly an important effect from the environment on the immune self that in turn affects the immune response. All of these observations favor the liquid self hypothesis. I will elaborate a little more on infection and cancer in Chapter 3.

Pregnancy is believed to be a tolerant state because the fetus is not being rejected. However, we always only consider successful pregnancies when making this assessment. It is possible that at least some unsuccessful fertilizations, implantations, and pregnancies represent in some way the effects of an intolerant state. Moreover, it is not uncommon for sensitization, with the development of anti-HLA antibodies, to occur as a result of pregnancy (the other two common causes being blood transfusions and previous transplants). Even one blood transfusion can result in profound sensitization, making it extremely difficult to perform a transplant afterwards. Conversely, while it is extremely difficult, if not impossible to conceive with end-stage kidney disease, a transplant will quickly restore fertility. Fertility is one of the expectations from a transplant. A wife being sensitized to a husband is much more common than vice versa, possibly owing to the main direction of transfer in antigen-containing fluids that takes place during repeated sexual intercourse. This can effectively prevent future husband-to-wife kidney donation. These are further examples of environmental influences on the immune self before transplantation.

After the transplant, however, pregnancy does not appear to increase the risk of rejection when the immune self has changed again.

Also, as mentioned before, HLA compatibility is not as important in kidney transplantation as it used to be, due to the great advances in anti-rejection medication, which is an environmental factor. Even blood type is becoming less relevant. Transplants can now be performed across blood type barriers without an undue amount of added immunosuppression. In fact, because the blood type "B" waiting list is so long in Ontario, some "A" kidneys are being allocated to them, without the need for anti-rejection medication more than normal. There has thus been spatiotemporal change in the way transplant medicine is practiced, and in the challenges being presented to transplant recipients at the population level and at the individual level.

The immune self therefore does not seem to be defined at conception, and it is not fixed at subsequent points in time either. So in summary, the liquid self hypothesis provides the best explanation for diverse post-transplant phenomena. It may even hold before the transplant. For example, a patient with a failed kidney transplant may have a negative cross-match against a potential donor for a second kidney transplant. If the first, failed transplant is then removed, the cross-match may turn positive, indicating the appearance of new pre-formed antibodies in the circulation. Thus the liquid self hypothesis can also be used to explain certain pre-transplant events.

The liquid self hypothesis can also be used to discuss where the self has its beginning and its end. Clearly, the self does not adhere strictly to the outline of the human body as we see in a drawing or a photograph. It is not the genome either. An identical twin, even though able to provide a kidney for transplantation, is not strictly the same because of epigenetic factors. The first kidney transplant was performed in 1954 between identical twins when only one twin developed the disease causing kidney failure in the first place. Thus, identical twins are not, in fact, strictly identical when more than the genome is taken

into account. Tolerance can be induced, at least in some experimental protocols, by the injection of bone marrow from the donor along with the kidney transplant, in order to induce tolerance by "incorporating" another human's genetic material into the immune self (chimerism). However, clinical trial results in this area are only somewhat encouraging.

Co-stimulatory blockade, once enthusiastically considered by Matzinger (2002) as a solution to rejection because it blocks additional lines of communication between the APC and T lymphocyte, has not fulfilled its initial promise in numerous clinical trials. There is still a lot of redundancy in the immune pathways that lead to rejection, since it can occur even when important pathways are blocked. Redundancy in the immune system pathways that leads to effective immune responses is more likely the result of spatiotemporal factors than anything fixed in the immune self.

A Combined Self-Nonself and Liquid Self Hypothesis

After combining these experiences from transplantation together and taking an overarching perspective, it appears that the self-nonself paradigm is still a very useful one, especially before the transplant and afterwards when things are going well. Transplantation becomes more challenging to perform as the self-nonself distinction is developed over the first few years of life. This can be best explained by the body's immune system "maturing" to generate a more effective immune response, but it may also be conceptualized as the result of the "sense of self" gaining strength as a result of a weakening in, i.e., less, tolerance.

In the later years of life, there is weakening of the immune surveillance system, as a result of which infection and cancer become more common because they are not being "rejected". With a transplant, the risk of these is enhanced even more, both as part of the natural propensity to these diseases anyway, and by the enormity of the new challenge thrown at the self by the transplant. Again, this could be an immune system phenomenon but instead, it may also be conceptualized that there is a weakening of the immune self so

that it cannot inform the immune system to defend it against other "selves" like infectious disease agents and cancer cells. The kidney is after all incorporated into the body's physiology in the same way as the native kidneys, so it would make sense that such incorporation would take place in the immune sense as well. The self-nonself theory thus still fits very well with clinical transplantation.

The liquid self hypothesis better explains why acute rejection sometimes develops suddenly and unexpectedly many years after transplantation. Also, pre-transplant autoimmune diseases like lupus may still remain active, leading to the sudden recurrence of the original disease in the graft. This would indicate that the self does indeed undergo change after the transplant. Either temporal or environmental changes have affected either the graft or the immune system, or both. Infections like CMV, an environmental factor, can cause acute rejection by altering the amount of HLA molecule expression in cells (van Dorp 1993). Even change in anti-rejection medication is an environmental change. We go from a more tolerant state to a less tolerant state as far as the transplant is concerned, and this progressively worsens over the course of life because the incidence of infection and cancer increases as well, while that of rejection does not decrease to the same extent. By analogous reasoning, this observation is also compatible with the psychological self being less able to handle sudden environmental change with advancing age.

At least in theory, the immune self can handle all internal body changes, including molecular changes. Variation in the pre-transplant anti-HLA antibody profile, with certain anti-HLA antibodies expressed at some times but not others, occurs so often that the antibody profile is checked several times a year while patients are waiting for a transplant, even when their apparent overall health has not changed. The anti-HLA antibody profile is checked so often because the most recent profile is given particular importance when selecting a donor. However, a memory response can always occur, so that remote anti-HLA antibodies can assume prominence post-transplant and cause acute rejection. It is quite possible that such "fluctuation" is the result of changes in the self-nonself status of the

foreign HLA inducing the antibodies, as a result of environmental influence. Many observations in clinical transplantation are therefore best handled by the liquid self hypothesis.

Conceptually, it is not difficult to recognize that the self indeed does react against the self in the healthy state. Self reacting against the self is a common occurrence, and essential to the health of the organism. Cells that are dying, or dead, are promptly phagocytosed through the triggering of immune effector functions (Savill 2002). Also, T regulatory cells downregulate activation of other lymphocytes and thereby regulate autoimmunity (Sakaguchi 2004). In fact, a weak reactivity to self-constituents is necessary in order for lymphocytes to survive in both the primary lymphoid organs (Ashton-Rickardt 1994) and secondary lymphoid organs (Freitas 1999). Reactions to the self are largely similar to those against non-self, harmful pathogens (Pradeu 2006). Despite the findings from the protocol biopsy studies, perhaps a bit of inflammation is "good" for the transplant, simply because it is an indicator that the immune system has decided to take the transplant under its protection, or the inflammatory process is a part of the larger assignment process of the transplant to self-status and therefore, incorporation into the larger self. The liquid self hypothesis handles the observation that self reacts against self innocuously, unlike the self-nonself theory, because the magnitude of this reaction may be determined by environmental influence. The liquid self hypothesis adequately explains that the immune self seeks to attain equilibrium, in harmony with the environment, although in reality it is never achieved. *The switch between self and nonself status for all bodily antigens is the essence of the continuing change in the immune self.*

To summarize, the five main theories of the self each explain some of the common observations of clinical kidney transplantation. It does not seem necessary to develop a separate transplant-based theory of the immune self. Dichotomization of the organism's parts into the so-called "self" and "nonself" is very useful for clinical purposes, especially before the transplant. Avoidance of sensitizing events like blood transfusions provides

clear benefits for subsequent transplants. The self-nonself paradigm provides a mechanism for matching kidney donors to recipients. It helps to minimize the risk of acute rejection, which can be quite harmful to the graft. However, many post-transplant observations, such as the inherent unpredictability of acute rejection, graft loss, infections, and cancer and the interactions among them; the change in transplant rejection risk over a lifetime; and autoimmune disease recurrence in the graft all point towards the liquid self hypothesis having greater explanatory power.

Although the immune system can be characterized mechanistically as systems of parts whose interactions produce regular changes that perform a biological function, the same cannot be said about the immune self. However, I do not believe that the inadequacy (so far) of the theories and hypotheses of the mechanism of the immune self and nonself distinction indicates that they are not explanatorily useful terms. Many clinical phenomena in transplantation (and other areas of medicine) are explainable by the self-nonself distinction.

Clinicians gauge the success of a transplant by the lack of any rejection response or significant post-transplant complications, which are immune responses. The overall success of the transplant is thus indicated by how well the immune self was able to adjust to the change induced by the transplant.

Chapter 3

Transplantation and Complications of the Immune Self

3.1 The Transplant in an Alien Environment

Transplantation often results in complications. It is clear that immune complications like infection and cancer are the result of alteration in the immune response, but I claim that these are also the result of a disruption in the immune self. I am also claiming, perhaps more provocatively, that many of the so-called "non-immune" complications of transplantation such as hypertension, diabetes, and excess weight gain (Danovitch 2005), to name only a few, are also misrepresentations in the self's attempt to evolve. In the case of a real transplant patient, unlike in the immunology laboratory, there are these and other diseases to address. Some of these diseases were present before the transplant, and some develop afterwards. We do not normally think of these as immune diseases. Each of these diseases has its own natural history, along the course of which there may be an effect on, or interaction with, the immune system however remote.

I propose that the adverse effects and other complications attributed to the transplant and the medications that accompany it are the result of the body failing to incorporate the new challenges that a transplant brings along with it. It was never nature's intention to deal with transplants. I propose that with any post-transplant disease, it is the immune self has become disrupted. The complications seen post-transplant are often attributed to the effects of the anti-rejection medications themselves (Table 2). The kidney itself experiences toxic effects from some of the anti-rejection medications. Kidney failure sometimes occurs when these medications are used for other organ transplants (Ojo 2003). Anti-rejection medications are blunt instruments that target the immune response. We do not yet have interventions to manipulate the immune self, because if we did. we would

create a true tolerant state in patients. True tolerance has not yet been achieved in transplantation.

The risk of acute rejection differs by organ. The skin, for example, is perhaps the most difficult organ to transplant, despite the apparently simple technical aspect to the procedure. Different organs require different amounts of medication to prevent acute rejection (for example, the liver requires less immunosuppression than the kidney, lung, or heart). Some side effects depend on the dose used, while others do not.

Table 2

Common side effects of anti-rejection medication

Calcineurin-inhibitor Medications (cyclosporine, tacrolimus)	**Other Medications (sirolimus, mycophenolic acid, azathioprine, prednisone)**
Toxicity to kidney	Delayed wound healing
Hypertension	Nausea and vomiting
High cholesterol	Diarrhea
Diabetes	Ankle swelling
Gout	Low white blood cells
Electrolyte imbalance	Low red blood cells
Tremor	Low platelets
Hair loss	Toxicity to liver
Excess hair growth	Excess weight gain
Infections	Cataracts
Cancer	Osteoporosis
	Infections
	Cancer

Anti-rejection medications are an important part of the environment of the transplant. With rare exceptions, there is no immune response to the medications themselves because they are not antigens. They cannot therefore be classified under either the self or nonself category. This is true for any environmental factor that does not interact with the immune system.

Tolerance to every currently transplantable organ still remains an unattainable goal. Multi-organ transplants are possible, such as a liver-kidney or heart-kidney transplant, and if the option is available, these organs are taken from the same donor in part to reduce the immune "load". However, if tolerance is the actual goal of the self throughout life, it follows that this should be its goal during the transplant process as well. To recollect, there are five main theories of the immune self. There is a self-nonself distinction being made, immune responses could be the result of tissue damage, immune networks may be operating throughout the body, continuity of molecular structure could be disrupted, and the self is acting as if it is liquid because it has no fixed spatiotemporal signature. These views may be equally compatible (or incompatible) with each organ. Rejection in each organ is also manifested differently, and is diagnosed in different ways. In the case of the kidney, recipient cells can be demonstrated to engraft in the transplanted organ by using sex-chromosome based karyotyping (Grimm 2001). The kidney thus becomes at least "part" self and its "foreignness" is correspondingly altered. Conversely, if a kidney is obtained from a living donor, it does not enlarge to the same extent in the recipient after the transplant, as the remaining kidney in the donor typically does after one kidney is removed. This discrepancy in what should be identical kidney size between donor and recipient is a reminder that the "nonself" status always remains. There could be a connection between the physiology of organ behavior, which can vary quite considerably by organ, and the immune system that is worth exploring. This may be the underlying basis for many non-immune complications of transplantation.

To better explain some post-transplant diseases, I will start by considering some basic facts about the kidney, since this particular organ is my focus. The goal of the kidneys is to preserve the equilibrium of the milieu interior, which was defined for us at the time our ancestors first took to land after having been in the primitive ocean up to that point. We are in effect carrying the primitive ocean in the form of the liquid content within our bloodstream, cells, and fluid between cells. Maintaining the precise concentration of substances in this fluid requires concentrating and diluting the urine as appropriate. What we do with the urine depends on whether we have too much or too little water in our body, and too much or too little salt (sodium) based on our highly variable dietary intake. Numerous other substances like other electrolytes (e.g., potassium, calcium, and magnesium, to name a few) as well as hydrogen ions are also controlled in this way. Many human diseases are the result of us having left our fish environment (Shubin 2008).

We do not have two kidneys for the sake of donating one of them. One hypothesis for why we have two kidneys is that in a state of volume depletion, when the urine is very concentrated, it is in a supersaturated state. Kidney stones may result, causing urinary tract obstruction and rapid failure of the blocked kidney. If we have two kidneys, the other kidney will take over. Potential kidney donors with a history of kidney stones are often turned down. In any chronic kidney disease (CKD), the remaining kidney enlarges and works harder to the point of becoming excessively strained, and fails more quickly, leading to ESKD. This seems to be an extremely counter-productive adaptation, but the apparent intention of nature is that fertility, which requires a certain level of kidney function, is to be preserved for as long as possible. Even if total lifespan for the individual is shortened as a result, the fertile period is lengthened as much as possible. Fertility is valued higher than long-term survival of the individual, because the population will benefit. Transplantation on the contrary often restores fertility, which nature did not expect, by resetting the time clock and facilitating further procreation.

There is also a normal aging process in the kidneys, with the result that approximately one percent of kidney function is lost per year after the age of 40. The histological changes in the kidney of normal aging are indistinguishable in many instances from chronic rejection, and so it is difficult to sort out what happened after the transplant, beyond what had already happened by the time a kidney that had already experienced some aging in the donor is implanted in the recipient. A kidney transplant is "like a used car" (Halloran 1999). Biopsies are always prone to sampling error and can be misleading. The kidney itself is a fused product arising from different embryological sources. The transplanted kidney does its best to perform all of the functions of the two native kidneys whose function it is replacing, such as those described above, as well as others like making hormones and maintaining blood pressure (BP). However, it has to do so in a new, alien environment, having just been taken out of its own, familiar environment of the donor. Most often, the two native kidneys are still in place and working, albeit to a far lesser extent than normal. A patient with a kidney transplant thus usually has three working kidneys. The transplanted kidney is a bit different from the two native kidneys though, by virtue of being in a separate anatomical location and receiving a different blood supply, and without nerve supply. The kidney transplant may also be of a significantly different age. Success in the whole endeavor is evidenced by the fact that it is not easy to distinguish between someone who has a working transplant and a normal, healthy person with two kidneys at many levels, including by blood tests. However, the transplant kidney is not capable of enlarging to the same extent as the remaining native kidney in the living donor because it is an alien environment. It also ages more quickly in the alien environment (Halloran 1999). Patients with kidney transplants are classified as being in just another stage of CKD. It makes sense then that complications are bound to eventually arise.

As I mentioned, among the well-described complications of transplantation are the so-called "immune" complications and the "non-immune" complications (Danovitch 2005). This division is broad but useful. Immune complications include conditions that

purportedly develop in the recipient as a result of immune system suppression, or the failure to suppress it. One common condition in this category that we have already discussed at length is acute rejection. When this happens, the upper hand in the self-nonself battle belongs to the host's immune response. The organ quickly becomes dysfunctional due to the body's determination to destroy it. Invading cells send out cytokines and other chemical substances allowing cells such as lymphocytes to actively seek out and destroy any tissue identified as "foreign". Antibodies get involved as well. The converse is GVHD, observed in bone marrow transplants. However, as I have discussed, it is quite possible that both rejection and GVHD are nothing but manifestations of a process of evolution in the character of the immune self that is behind the immune response, due to the challenge from the environment. It is a clash of two "selves". The important point to note here is that other organ systems can become involved in the process. Although in Chapter 2, I had mentioned that acute rejection is often asymptomatic, there can still be systemic effects. For example, the BP may rise, or diabetes may develop. In the case of GVHD, the skin and gut are often affected.

3.2 Transplant Complications from the Immune Self Perspective

We will now discuss selected post-transplant complications and try to characterize them as alterations in the immune self. I argue that the complications seen after transplantation can be related to, and are further evidence of the immune self's ongoing evolution. The difficulty or inability to address the challenge provided by transplantation to incorporate new information from the graft into the sense of self is what leads to these complications. The self's ultimate goal is to be in a state of equilibrium with its internal and external environment, and this can be loosely understood as "health" in its broadest sense. Even if the graft is "accepted", there may be systemic consequences in order for it to be accepted. These consequences may include what physicians and patients normally

consider to be diseases, and are therefore undesirable. However, they may be unavoidable, and even an essential part of the transplant recipient's health.

I will first discuss two common immune complications of transplantation, infection and cancer. Following this, I will discuss two non-immune complications, hypertension and obesity. Although there are others, I have chosen these because they are both common and have important consequences. All of these are viewed through the lens of the immune self.

Infection

Infection is the classic folk example of immune imbalance, since in lay terminology "resistance" to infection is equated with health. The risk for infection is often compounded by anti-rejection medications. Unfortunately, as stated before, the actual level of immunosuppression achieved in a given patient is impossible to quantify because there is no scale or instrument for its measurement. Physicians use rough guides such as anti-rejection medication dose (which is often not even titrated to body weight), or blood levels which are simply snapshots in time and do not necessarily reflect overall drug exposure. The type of infection is varied, dependent on both the extent of immunosuppression and the time that has elapsed since the transplant.

Microbial residence does not necessarily imply that there is infection. There are non-self microbes that reside in us, do not always cause harm, and may in fact be quite beneficial. In the case of severe overgrowth of one type of harmful bacterium, *Clostridium difficile*, fecal transplants have been performed in the case of severe and recurrent diarrhea (van Nood 2013). In this case, the "self" can be easily conceptualized to consist of the body and all the commensals and parasites contained within it. The large microbial community that colonizes a host such as a human being is called the "microbiota" (Alegre 2014). It has been estimated that there are as many as 10^{14} commensal microorganisms in the gut (Pradeu 2006). Although the greatest concentration of microbes (including viruses, bacteria, parasites, and fungi) is greatest in the large intestine, microbes are also present on the

skin, in other parts of the gastrointestinal tract including the mouth and oropharynx, and in the genitourinary tract, reproductive tract, and respiratory tract.

Microbes perform a number of useful, indispensable functions. One important function of these microbes is to break down complex carbohydrates in the gut to provide free fatty acids to serve as an energy source for gut epithelial cells and to serve as a substrate for the synthesis of glucose and lipids (Tremaroli 2012). The microbiota also functions to help with the reabsorption of secreted bile acids, synthesize vitamin K, and absorb amino acids (Alegre 2014). By competing with harmful microbes ("pathobionts") for nutrients, they help prevent infections that may be harmful to the host. In addition, microbiota also help facilitate maturation of the host's immune system. Their help ensures normal functioning of gut-associated secondary lymphoid tissue, the generation of immunoglobulin-secreting B cells, and differentiation of induced regulatory T cells (Alegre 2014). Although the effect of these microbes has been largely thought to be local, it may in fact be applicable to distant parts of the body, *including grafts.*

Interestingly, although the exact organisms that constitute the microbiota may vary from one individual body to the next, the sum total of its genetic material (the "metagenome") is remarkably constant (Human Microbiome Project Consortium 2012). A possible consequence of the increased hygiene and less microbial exposure over the past century of human development may be an increased incidence of autoimmune diseases, as a result of the altered microbiota. This is part of the so-called "hygiene hypothesis" (Rook 2012).

The role of the microbiota in transplant recipients is still in a very early stage of investigation (Alegre 2014). We do not know the composition of the microbiota in the recipient at the time of the transplant. Although the transplanted kidney itself is considered sterile, the deceased donor may have an infection and the risk for infection transmission is always present. In GVHD, the severity of the condition may be dictated by

the extent of the alteration in the normal composition of the microbiota ("dysbiosis") and the proliferation of harmful bacteria like *Escherichia coli* (Eriguchi 2012). It has been demonstrated that there is a substantial alteration in the microbiota after kidney transplantation (Fricke 2014). This finding alone is sufficient to demonstrate that the self, in its widest sense, is altered or even disrupted by the process of transplantation. The use of prophylactic antibiotics, which is a common practice early after transplantation, may result in the proliferation of harmful bacteria at the expense of useful bacteria in some transplant populations (Wu 2012). Small bowel transplants are quite difficult to perform because of numerous complications. It has been shown that an altered microbiota is associated with rejection of the transplant (Oh 2012).

Viral infections in particular are important early after the transplant, especially in the period between one and six months post-transplant. This period is when the level of immunosuppression is kept relatively high in order to prevent acute rejection, whose incidence is also high during that time period. Deoxyribonucleic acid (DNA) viruses like CMV and EBV reside dormant in the body long after initial infection, and sometimes cause trouble in the early post-transplant period. Most people have expanded clones of T lymphocytes specific for CMV and EBV (Vescovini 2004). The immune response normally keeps these infections in check.

The occurrence of BKV nephropathy, which is a serious, specific viral infection of the kidney transplant, has been shown to correlate with a higher degree of HLA mismatches (Awadalla 2004). Primary infection from BKV usually occurs in childhood, with 50% of children having seroconverted for this virus by 3-4 years of age, but, along with a related virus called JC virus (also named after a patient) the prevalence of infection increases to 80% by adulthood (Pinto 2014). BKV is a DNA virus that normally resides in the urinary tract, and asymptomatic shedding of the virus in the urine is not uncommon even in non-transplant populations. BKV DNA can also be measured in the blood. Within about three months after transplantation, the virus reactivates in 30-50% of kidney transplant

recipients (Bressollette-Bodin 2005, Brennan 2005). BKV is detectable in the urine in about 80% of all patients while 5-10% go on to develop BKV nephropathy (kidney disease). A major risk factor for viral reactivation is excessive immunosuppression, as can happen after acute rejection has been treated. So a means for treating this infection is to reduce anti-rejection drugs (Brennan 2005) but this does not always work. If the infection spreads to the kidney with destruction of kidney cells and progressive scarring, then graft failure occurs in 50-80% of patients (Pinto 2014).

In the case of BKV nephropathy, the immune self has failed to maintain a state of equilibrium with BKV after the transplant. This equilibrium obviously existed before the transplant because there was no BKV nephropathy in the native kidneys. Perhaps the virulence of BKV increases in the context of a now-alien environment. The immune system has failed to contain BKV. However, interpreted differently, BKV has switched from self to nonself status. The virus does not seem to be content until the kidney is destroyed, even though it will die out on the process (unlike in the case of rabies, for example, where host aggressiveness will help to find another host for the virus). The native organs are not affected. The immune response always needs to be kept in fine balance between causing acute rejection on one hand, and allowing viral infections like BKV to proliferate on the other, but when one becomes a risk factor for the other, the immune system has clearly lost this capability. In some patients this balance is very delicate, while in others BKV nephropathy never occurs even if very powerful anti-rejection therapy is used. This indicates the possibility that there is something beyond just the immune response determining equilibrium, possibly the immune self.

BKV nephropathy is a disease of modern times; it was never encountered in Burnet's era. The phenomenon is generally attributed to more intensive routine immunosuppression in the modern era. The immunological biography (Grignolio 2014) has thereby been altered in many transplant recipients, and therefore at a population level as well.

Cancer

Cancer, the second so-called "immune" complication, is also common after a transplant. We have already briefly reviewed skin cancer and Kaposi's sarcoma. In this section I will focus on lymphoma after the transplant, because in this case there is an important crossroad between infection and cancer, thereby effectively illustrating the central importance of the immune self.

EBV infection is associated with a serious complication called post-transplant lymphoproliferative disorder (PTLD) (Green 2013), which is a type of lymphoma (cancer) that can sometimes be fatal. In its most benign form, the virus causes infectious mononucleosis in teenagers. About ninety-eight percent of people have been exposed to EBV. In the case of transplantation, if exposure to the virus has not occurred yet, the virus may be acquired from a donor who has previously been exposed to the virus. This situation is called an "EBV mismatch" and represents a high-risk situation for the development of PTLD. Fortunately, about 90-95% of adults are seropositive for EBV (Green 2013), and so are at a lower risk for PTLD, regardless of the donor's status. Special precautions are often taken to minimize the risk of PTLD in recipients at higher risk, including using less anti-rejection medication than normal.

EBV persists in B lymphocytes, with any attempt at an active infection quickly suppressed by cytotoxic T lymphocytes. It has been estimated that there is one virus particle in every 10^6 B lymphocytes in most adults (Green 2013). Once immunosuppression occurs after a transplant, any B cells infected with EBV that come along with the graft do not elicit the same response from the cytotoxic T lymphocytes, especially when immunosuppressive drugs directed against them are used in high doses. The lymphocytes (B or T) begin to proliferate and cause lymphoma. If the lymphocytes all come from one clone, the prognosis for the patient is much worse. PTLD can occur in multiple organs and can cause death.

In PTLD, the effector arm of the immune response has gone awry. The immune response is destroying the body because all tolerance to self and nonself has been lost. Exposure to EBV as a teenager to a youthful immune self seems harmless because of the type of immune response it elicits, but exposure to an immune self that has just been altered by transplantation can be devastating because of the type of immune response it fails to elicit. Thus, the harm caused by EBV is strongly dependent on the circumstance under which infection occurs. This variation in effect is a very powerful example that supports the liquid self hypothesis and argues against the self-nonself theory, because teenagers already have passed the point of transplantation without immunosuppression but at the same time are not significantly harmed by EBV. The liquid self hypothesis, by virtue of being an extension of the danger theory, provides the explanation that the danger posed by early stage cancers is no longer being recognized by the immune self, resulting in cancer proliferation as a result of spatiotemporal change in the recipient. The effect of the altered immune self on cancer risk is very difficult to predict at the individual level.

Screening for common forms of cancer before a transplant is very important, not only because an existing cancer may proliferate after the transplant, but the risk for all cancers may be increased in general. An immune self (and immune response) that has allowed a cancer to develop once before may do so again. If anti-rejection medication is given in large enough doses for a long enough period of time, then just about anyone will develop cancer. Transplantation also represents the only situation in which a cancer is artificially introduced into the body, albeit inadvertently. With immunosuppression, such cancers can then proliferate. In the case of EBV mismatch, patients are counseled about the risk of PTLD before the transplant. Some patients then choose not to proceed. Cancer is a particularly severe manifestation of the immune self's regulatory process failure. The intersection of infection and cancer in the case of EBV shows how the immune self is intricately linked to both. The wide variability in cancer risk and outcomes illustrates how the self-status of cancer cells may be altered in the liquid self.

Hypertension

Hypertension has a genetic component and an environmental component. It is a very common disease in the general population. If it is to qualify as a result of disruption in the immune self, then at minimum it must be caused or worsened by transplantation. It should also have an immune component, in order to be the result of the immune self's failure to readjust after the transplant.

A high BP causes heart attacks and strokes. It is of particular interest in the post-transplant setting, not only because it is intimately linked to CKD, but because it is also a very common disease even when not linked to CKD. Hypertension can occur for the first time after a transplant, and might be transmissible along with the kidney from donor to recipient. It can even make the transplant more immune reactive (Pratschke 2004). New antigens may be generated as a result of elevations in BP due to damage to the blood vessels (Schiffrin 2012). APC then adopt a pro-inflammatory phenotype, migrate to secondary lymphatic tissue, and stimulate lymphocytes called Th1 effector lymphocytes. These produce harmful cytokines like gamma interferon and interleukin-6. A different group called Th17 effector lymphocytes produces another cytokine called interleukin-17 (Schiffrin 2013). Thus it has been proposed that hypertension leads to inflammation, which is also a hallmark of transplant-related immune processes like acute rejection. High BP is believed to be caused by rejection, but it is also possible that the reverse is the case (Cosio 2001). A high BP can precede acute rejection. These findings indicate the close relationship between BP and the immune response.

Dendritic cells, NK cells, and activated B and T lymphocytes are all found in the fat tissue surrounding blood vessels. Similarly, macrophages (a type of WBC) representing the innate immune system are found, and the combination of adaptive and innate immunity is what is believed to lead to the pathophysiology of hypertension (Verlohren 2009, Harrison 2011). A high BP is very common soon after the transplant, when one can speculate that it

is actually a marker of the immune self's adjustment process. High BP is a reliable marker of poor graft function (Opelz 1998), and possibly poorer patient survival. It is rejection however that is most harmful, and most acute and chronic rejection is linked in some way to high BP. BP then becomes a marker of graft performance. It is not exactly an "immune meter" but it is close to being one for the kidney graft. The blood vessel leading to the transplant can also be rejected, leading to its narrowing. High BP can occur as a result. A high BP without obvious cause including an overt immune response, could, albeit speculatively, be related to the immune self causing an evolution of normal immune reactivity into an immune response. If hypertension is indeed an immune disease, then it may be a disorder of the immune self after a transplant.

High BP can also be a marker of poor patient adherence, thus linking it to the psychological self as well. Much emphasis is placed on BP measurements in the clinic setting, and many patients are acutely aware of their BP readings, having associated high BP with the failure of their native kidneys long before receiving the transplant.

Obesity

Our final post-transplant condition, obesity or excess body weight, is often linked to hypertension and diabetes, as part of the so-called metabolic syndrome after transplantation. It is quite common for patients to gain a significant amount of weight soon after receiving a transplant. Some anti-rejection drugs like prednisone stimulate the appetite, but also, patients feel much better after being relieved of ESKD symptoms such as nausea. For obvious reasons, obesity has a marked impact on the psychological self. However, obesity has also been linked to many immune processes (Heinbokel 2013).

Two important hormones that have a relationship to obesity are "adiponectin" and "leptin". Blood levels of adiponectin are decreased in the presence of obesity, when fat tissue envelops the internal organs (Arita 1999). Adiponectin leads to a muted T lymphocyte response to antigens (Wolf 2004) and may therefore result in less transplant

rejection (Okamoto 2009). Adiponectin also inhibits the production of pro-inflammatory cytokines such as gamma interferon and tumor necrosis factor-alpha in macrophages (Wolf 2004). It can also inhibit the development of B cells by activating prostaglandins, which are compounds associated with inflammation (Yokota 2003)

Unlike adiponectin, leptin has a pro-inflammatory function, favoring the production of CD4+ T cells in response to blood cells transfused from another person. Thus, leptin promotes the adaptive immune response (Lord 1998). Leptin also increases the innate immune response by increasing the phagocytic ability of both monocytes (yet another type of WBC) and macrophages (Zarkesh-Esfahani 2001). Leptin stimulates the ability of phagocytes to kill bacteria (Caldefie-Chezet 2001) and it also promotes the cytotoxicity of NK cells (Tian 2002). At a clinical level, obesity is a well-known risk factor for transplant failure (Meier-Kriesche 2002), and at least one study has shown an association with an increased rate of acute rejection in obese patients (Gore 2006). Obesity, by virtue of being an environmental influence may affect the immune self-nonself status of particular antigens such as graft antigens, and not just the psychological self.

To summarize Chapter 3, a closer look at some common complications of kidney transplantation still allows for dichotomization of the organism's parts into the so-called "self" and "nonself". The immune system itself is involved in many of these complications. The four post-transplant diseases I reviewed in the specific context of kidney transplantation (infection, cancer, hypertension, and obesity) each has intimate links to the immune system. The changed environment after a transplant can permit any of these diseases to emerge. Each disease also has significant consequences for the graft. While these complications are typically viewed from the standpoint of an altered immune response or non-immune pathway, it is at least a possibility that they are instead the result of variation induced in the immune self. This is because of the wide variability seen in the type and severity of complications seen with the same anti-rejection medications. This

observation is compatible with the liquid self hypothesis, and supports my claim that the liquid self hypothesis has greater explanatory power for a variety of post-transplant events.

It is possible that many diseases that occur after transplantation were not caused by "the transplant" directly but were likely to occur anyway. However, the natural history of such diseases has certainly been accelerated by transplantation, and the clinical emergence of these diseases was thus brought forward to an earlier age in the lifespan of the individual, because the immune self has now been recalibrated by the presence of a graft. Thus, to state that a transplant "caused" such-and-such a disease would be a misrepresentation of the entire transplant process. The immune self would have eventually permitted these diseases to emerge at some point in later life. The new immune self has been redefined by the transplant. Post-transplant complications are not only the result of the new environment created by the graft, but also actively contribute towards defining that environment. In the case of complications, the prevailing disease-suppressing conditions have now become disease-promoting.

Chapter 4

Summary and Conclusion

4.1 Summary

In my thesis, I have focused on the immune self, which is a component of the molecular self, in order to understand the nature of the self. I have used the experience of clinical kidney transplantation to analyze five major prevailing theories surrounding the nature of the immune self. Among these, the self-nonself theory provides an excellent paradigm to help guide the extensive pre-transplant preparations that are required to make the transplant a success. HLA molecules are identified in the donor and recipient to help facilitate donor-recipient selection and cross-matching. Anti-HLA antibodies are detected in the recipient. Knowledge of any self-nonself discrepancy provides a rationale for the use of specific anti-rejection drugs to counter the potential immune response. However, the self-nonself theory is clearly inadequate when attempting to analyze the common complications seen after a transplant. Complications like acute rejection still occur even after very close matching. The graft can function very poorly even when nothing is seen on biopsy. The type and severity of complications cannot be easily predicted even with such a thorough pre-transplant assessment.

Some of the other theories of the immune self do not perform much better in the post-transplant setting. The danger theory is difficult to comprehend in this context because the kidney is not an immunogenic organ and does not attack the recipient. The danger theory would imply that the immune self consistently misreads the graft, which has nothing dangerous about it. Even if biochemical danger signals are being emitted from the graft, as a result of surgical manipulation, such signals are released from damaged native tissue as well and they do not cause acute rejection. The immune network theory, which provides an excellent explanation for the occurrence of autoimmune disease, is not helpful

in transplantation because autoimmune diseases do not have appreciable consequences for transplantation and the transplant never really is “auto”. An immune response cannot be an extension of autoreactivity. Lymphocytes are clearly invading the graft, so it is not a disruption in communication because none existed before. The continuity theory is disadvantaged by the vagueness of molecular difference, and perhaps the naïve supposition that the self, even if it is evolving, is relatively static when it encounters a molecular change. The immune self may also be independently changing whether it meets the stimulus or not.

After the transplant, the liquid self hypothesis appears to hold the most explanatory power. The immune self is fundamentally and suddenly transformed by transplantation. The microbial environment undergoes change. The expression of HLA molecules on the surface of cells changes as well. Most importantly, there is a new kidney full of unfamiliar antigens, posing the challenge of a conversion of normal immune reactivity into a full-fledged immune response. These phenomena are all consistent with the ever-evolving nature of the self. Transplantation provides us with an opportunity to examine a sudden, recognizable change in the self. This change occurs at the individual, social, neural, and molecular levels. The variable nature of the immune (and non-immune) response to transplantation is an indicator of the fluctuation in the nature of the self over time. This change also indicates that the immune self cannot be tied down to a single molecular signature, and is most likely related to the environment in which it finds itself, even if did not exist before in that environment. Two kidneys from the same donor transplanted into two different recipients will have two different outcomes. Acute rejection is a transplant-specific and individual-specific process that exists because the graft has been assigned nonself status by the immune self. Some transplant patients develop acute rejection as an immune response while others do not because the transplant has been assigned nonself status at the time acute rejection commences.

When conditions such as infection, cancer, hypertension, and obesity are viewed through the lens of the immune self, the immune-nonimmune classification of complications loses significance. The liquid self hypothesis provides the explanation that the immune self is altered after the transplant, in turn altering the natural history of these conditions.

Tauber states that "a resting point never exists and never arrives" (Tauber 1994). The immune system may be slowly evolving towards some goal (which I have called "equilibrium") that is never reached, or it may be a relatively stable entity punctuated by interruptions. In either case, it is always changing.

4.2 Conclusion

I have asked several questions regarding the nature of the immune self. In this concluding section I will now summarize my answers. The experience of clinical kidney transplantation fits well with several of the existing theories of the immune self, so a distinct theory is not required. The immune self is a real entity because it provide an explanation for real phenomena like acute rejection beyond what the immune response alone can provide. Correspondingly, there is such a thing as an immune nonself, because it is the graft which is clearly the target of the immune response after a transplant. The role of the immune self is to achieve the body's equilibrium between tolerance and rejection of individual antigens, although in reality this is never achieved. The immune self is constantly undergoing change as part of this process. The success of a transplant, which is possibly the most significant change imposable on the immune self, is an indicator of how well the immune self was able to adjust to the transplant.

Existing theories of the immune self are helpful but not sufficient to explain known transplant phenomena, although the self-nonself theory and the liquid self hypothesis seem to have the greatest explanatory value before and after the transplant respectively. The self-nonself theory provides a good account of how the immune self functions when

events evolve as predicted, which is typical of the pre-transplant situation, assuming of course that the least disruption to the immune system possible is ideal for health. The liquid self hypothesis provides a good account when events do not evolve as predicted, which is typical of the post-transplant situation where complications are inevitable. Having an immune system is not enough for acute rejection and numerous other phenomena; its reason is provided by the immune self. My hypothesis, which is that transplantation shows us that the immune self is ever changing and that the success of transplantation is determined by how well the immune self adapts to the challenge that a transplant imposes, is supported by the experience of clinical transplantation.

Although the tendency of each individual to express certain antigens and not others is pre-determined, every immune self gets defined and redefined based on the experience from its encounters. Our immunosuppressive therapies are designed to suppress the immune response, but the better target is perhaps the switch that determines the self-nonself status of particular antigens. The immune self should be viewed as the target of transplant physicians, to be cared for and nurtured, since it is the seat of all the diseases, disorders, and complexities encountered at all stages in the transplant process.

References

Adkins B, Leclerc C, Marshall-Clarke S (2004) Neonatal adaptive immunity comes of age. *Nature Reviews Immunology* 4:553-564.

Alegre M-L, Mannon RB, Mannon PJ (2014) The microbiota, the immune system and the allograft. *American Journal of Transplantation* 14:1236-1248.

Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shiromura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochemical and Biophysical Research Communications* 257:79-83.

Ashton-Rickardt PG, Bandeira A, Delaney JR, Van Kaer L, Pircher HP, Zinkernagel RM, Tonegawa S (1994) Evidence for a differential avidity model of T cell selection in the thymus. *Cell* 76:651-663.

Awadalla Y, Randhawa P, Ruppert K, Zeevi A, Duquesnoy RJ (2004) HLA mismatching increases the risk of BK virus nephropathy in renal transplant recipients. *American Journal of Transplantation* 4:1691-1696.

Baedke J (2013) The epigenetic landscape in the course of time: Conrad Hal Waddington's methodological impact on the life sciences. *Studies in History and Philosophy of Biological and Biomedical Sciences* 44:756-773.

Blackmore S (2012) Consciousness: An Introduction, Second Edition, Oxford University Press, New York, NY, USA.

Brennan DC, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, Lockwood M, Torrence S, Schuessler T, Roby T, Gaudreault-Keener M, Storch GA (2005) Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *American Journal of Transplantation* 5:582-594.

Bressollette-Bodin C, Coste-Burel M, Hourmant M, Sebille V, Andre-Garnier E, Imbert-Marcille BM (2005) A prospective longitudinal study of BK virus infection in 104 renal transplant recipients. *American Journal of Transplantation* 5:1926-1933.

Burnet FM (1957) A modification of Jerne's theory of antibody production using the concept of clonal selection. *Australian Journal of Science* 20:67-69.

Burnet FM (1991) The Nobel Lectures in Immunology. The Nobel Prize for Physiology or Medicine, 1960. Immunologic recognition of self. *Scandinavian Journal of Immunology* 33:3-13.

Caldefie-Chezet F, Poulin A, Tridon A, Sion B, Vasson MP (2001) Leptin: a potential regulator of polymorphonuclear neutrophil bactericidal action? *Journal of Leukocyte Biology* 69:414-418.

Claverie JM, Kourisky P (1986) The peptidic self-model: a reassessment of the role of major histocompatibility molecules in the restriction of the T-cell response. *Annales de l'Institut Pasteur Immunology* 137D:425-442.

Cosio FG, Pelletier RP, Pesavento TE, Henry ML, Ferguson RM, Mitchell L, Lemeshow S (2001) Elevated blood pressure predicts the risk of acute rejection in renal allograft recipients. *Kidney International* 59:1158-1164.

Danovitch GM (2005) Handbook of Kidney Transplantation, Fourth Edition. Lippincott Williams and Wilkins, Philadelphia, PA, USA.

Eriguchi Y, Takashima S, Oka H, Shimoji S, Nakamura K, Uruyu H, Shimoda S, Iwasaki H, Shimono N, Ayabe T, Akashi K, Teshima T (2012) Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of α-defensins. *Blood* 120:223-231.

Franceschi C, Bonafe M, Valensin S, Oliveri F, De Luca M, Ottaviani E, De Benedictis G (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. *Annals of the New York Academy of Sciences* 908:244-254.

Freitas AA, Rocha B (1999) Peripheral T cell survival. *Current Opinion in Immunology* 11:152-156.

Fricke WF, Maddox C, Song Y, Bromberg JS (2014) Human microbiota characterization in the course of renal transplantation. *American Journal of Transplantation* 14:416-427.

Gore JL, Pham PT, Danovitch GM, Wilkinson AH, Rosenthal JT, Lipshutz GS, Singer JS (2006) Obesity and outcome following renal transplantation. *American Journal of Transplantation* 6:357-363.

Green M, Michaels MG (2013) Epstein-Barr virus infection and posttransplant lymphoproliferative disorder. *American Journal of Transplantation* 13:41-54.

Grignolio A, Mishto M, Faria AMC, Garagnani P, Franceschi C, Tieri P (2014) Towards a liquid self: how time, geography, and life experiences reshape the biological identity. *Frontiers in Immunology* 5:153

Grimm PC, Nickerson P, Jeffery J, Savani RC, Gough J, McKenna RM, Stern E, Rush DN (2001) Neointimal and tubulointerstitial infiltration by recipient mesenchymal cells in chronic renal-allograft rejection. *New England Journal of Medicine* 345:93-97.

Haas M (2014) Chronic allograft nephropathy or interstitial fibrosis and tubular atrophy: what is in a name? *Current Opinion in Nephrology and Hypertension* 23:245-250.

Halloran PF, Melk A, Barth C (1999) Rethinking chronic allograft nephropathy: The concept of accelerated senescence. *Journal of the American Society of Nephrology* 10:167-181.

Harrison DG, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, Vinh A, Weyand CM (2011) Inflammation, immunity, and hypertension. *Hypertension* 57:132-140.

Heinbokel T, Floerchinger B, Schmiderer A, Edtinger K, Liu G, Elkhal A, Tullius SG (2013) Obesity and its impact on transplantation and alloimmunity. *Transplantation* 96:10-16.

Ho J, Wiebe C, Gibson IW, Rush DN, Nickerson PW (2012) Immune monitoring of kidney allografts. *American Journal of Kidney Diseases* 60:629-640.

Human Microbiome Project Consortium (2012) Structure, function, and diversity of the healthy human microbiome. *Nature* 486:207-214.

Huurre A, Kalliomaki M, Rautava S, Rinne M, Salminen S, Isolauri E (2008) Mode of delivery-effects on gut microbiota and humoral immunity. *Neonatology* 93:236-240.

Jerne NK (1974) Towards a network theory of the immune system. *Annales d'immunologie* 125C:373-389.

Joffre O, Nolte MA, Sporri R, Reis E, Sousa C (2009) Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. *Immunological Reviews* 227:234-247.

Kawai T, Sachs DH, Sprangers B, Spitzer TR, Saidman SL, Zorn E, Tolkoff-Rubin N, Preffer F, Crisalli K, Gao B, Wong W, Morris H, LoCascio SA, Sayre P, Shonts B, Williams Jr. WW, Smith R-N, Colvin RB, Sykes M, Cosimi AB (2014) Long-term results in recipients of combined HLA-mismatched kidney and bone marrow transplantation without maintenance immunosuppression. *American Journal of Transplantation* 14:1599-1611.

Lamb KE, Lodhi S, Meier-Kriesche H-U (2011) Long-term renal allograft survival in the United States: A critical appraisal. *American Journal of Transplantation* 11:450-462.

Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 394:897-901.

Mackay IR, Larkin L, Burnet FM (1957) Failure of autoimmune antibody to react with antigen prepared from the individual's own tissues. *Lancet* 273:122-123.

Markus H, Kunda Z (1986) Stability and malleability of the self-concept. *Journal of Personality and Social Psychology* 51:858-866.

Matzinger P (1994). Tolerance, danger, and the extended family. *Annual Review of Immunology* 12:991-1045.

Matzinger P (2002) The danger model: A renewed sense of self. *Science* 296:301-305.

Meier-Kriesche H-U, Arndorfer JA, Kaplan B (2002) The impact of body mass index on renal transplant outcomes: a significant independent risk factor for graft failure and patient death. *Transplantation* 73:70-74.

Meier-Kriesche H-U, Chu AH, David KM, Chi-Burris K, Steffen BJ (2006) Switching immunosuppressive medications after renal transplantation-a common practice. *Nephrology Dialysis Transplantation* 21:2256-2262.

Narayanan R, Cardella CJ, Cattran DC, Cole EH, Tinckam KJ, Schiff J, Kim SJ (2010) Delayed graft function and the risk of death with graft function in living donor kidney transplant recipients. *American Journal of Kidney Diseases* 56:961-970.

Oh PL, Martinez I, Sun Y, Walter J, Peterson DA, Mercer DF (2012) Characterization of the ileal microbiota in rejecting and non-rejecting recipients of small bowel transplants. *American Journal of Transplantation* 12:753-762.

Ojo AO, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, Arndofer J, Christensen L, Merion RM (2003) Chronic renal failure after transplantation of a nonrenal organ. *New England Journal of Medicine* 349:931-940.

Okamoto Y, Christen T, Shimizu K, Asano K, Kihara S, Mitchell RN, Libby P (2009) Adiponectin inhibits allograft rejection in murine cardiac transplantation. *Transplantation* 88:879-883.

Opelz G, Wujciak T, Ritz E (1998) Association of chronic kidney graft failure with recipient blood pressure. Collaborative Transplant Study. *Kidney International* 53:217-222.

Pennington R, Gatenbee C, Kennedy B, Harpending H, Cochran G (2009) Group differences in proneness to inflammation. *Infection Genetics and Evolution* 9:1371-1380.

Pinto M, Dobson S (2014) BK and JC virus: A review. *The Journal of Infection* 68:52-58.

Pradeu T, Carosella ED (2006) On the definition of a criterion of immunogenicity. *Proceedings of the National Academy of Sciences of the United States of America* 103:17858-17861.

Pradeu T (2012a) The limits of the self: immunology and biological identity, Oxford University Press, New York, NY, USA

Pradeu T, Cooper EL (2012b) The danger theory: 20 years later. *Frontiers in Immunology* 3:287

Prugnolle F, Manica A, Charpentier M, Guegan JF, Guernier V, Balloux F (2005) Pathogen-driven selection and worldwide HLA Class I diversity. *Current Biology* 15:1022-1027.

Pratschke J, Paz D, Wilhelm MJ, Laskowski I, Kofla G, Vergopoulos A, MacKenzie HJ, Tullius SG, Neuhaus P, Hancock WW, Volk HD, Tilney NL (2004) Donor hypertension increases graft immunogenicity and intensifies chronic changes in long-surviving renal allografts. *Transplantation* 77:43-48.

Rook GA (2009) Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. *Immunology* 126:3-11.

Rook GA (2012) Hygiene hypothesis and autoimmune diseases. *Clinical Reviews in Allergy and Immunology* 42:5-15.

Rush DN, Karpinski ME, Nickerson P, Dancea S, Birk P, Jeffery JR (1999) Does subclinical rejection contribute to chronic rejection in renal transplant patients? *Clinical Transplantation* 13:441-446.

Sakaguchi S, Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T (2009) Regulatory T cells: how do they suppress immune responses? *International Immunology* 21:1105-1111.

Sakaguchi S (2004) Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annual Review of Immunology* 22:531-562.

Savill J, Dransfield I, Gregory C, Haslett C (2002) A blast from the past: clearance of apoptotic cells regulates immune responses. *Nature Reviews Immunology* 2:965-975.

Schiffrin EL (2012) Vascular remodeling in hypertension: mechanisms and treatment. *Hypertension* 59:367-374.

Schiffrin EL (2013) The immune system: Role in hypertension. *Canadian Journal of Cardiology* 29:543-548.

Shubin N (2008) Your inner fish: A journey into the 3.5-billion-year history of the human body. Random House Inc., Pantheon House, New York, NY, USA.

Sironi M, Clerici M (2010) The hygiene hypothesis: an evolutionary perspective. *Microbes and Infection* 12:421-427.

Strachan DP (1989) Hay fever, hygiene, and household size. *British Medical Journal* 299:1259-1260.

Tauber AI (1994) The Immune Self: Theory or Metaphor? Cambridge University Press, New York NY, USA

Thagard P (2014) The self as a system of multilevel interacting mechanisms. *Philosophical Psychology* 27:145-163.

Tian Z, Sun R, Wei H, Gao B (2002) Impaired natural killer (NK) cell activity in leptin receptor deficient mice: leptin as a critical regulator in NK cell development and activation. *Biochemical and Biophysical Research Communications* 298:297-302.

Tonegawa S (1983) Somatic generation of antibody diversity. *Nature* 302:575-581.

Tremaroli V, Backhed F (2012) Functional interactions between the gut microbiota and host metabolism. *Nature* 489:242-249.

Vance RE. (2000) Cutting Edge Commentary. A Copernican revolution? Doubts about the danger theory. *Journal of Immunology* 165:1725-1728.

van Dorp WT, van Wieringen PA, Marselis-Jonges E, Bruggeman CA, Daha MR, van Es LA, van der Woude F (1993) Cytomegalovirus directly enhances MHC class I and intercellular adhesion molecule-1 expression on cultured proximal tubular epithelial cells. *Transplantation* 55:1367-1371.

van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ (2013) Duodenal infusion of donor feces for recurrent Clostridium difficile. *New England Journal of Medicine* 368:407-415.

Vaz NM, Varela FJ (1978) Self and non-sense: An organism-centered approach to immunology. *Medical Hypotheses* 4:231-267.

Verlohren S, Muller DN, Luft FC, Dechend R (2009) Immunology in hypertension, preeclampsia, and target-organ damage. *Hypertension* 54:439-443.

Vescovini R, Talera A, Fagnoni FF, Biasini C, Medici MC, Valcavi P, di Pede P, Lucchini G, Zanlari L, Passeri G, Zanni F, Chezzi C, Franceschi C, Sansoni P (2004). Different contribution of EBV and CMV infections in very long-term carriers to age-related alterations of CD8+ T cells. *Experimental Gerontology* 39:1233-1243.

West LJ (2011) ABO incompatible hearts for infant transplantation. *Current Opinion in Organ Transplantation* 16:548-554.

Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H (2004) Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochemical and Biophysical Research Communications* 323:630-635.

Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, Held PJ, Port FK (1999) Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *New England Journal of Medicine* 341:1725-1730.

Wu ZW, Ling ZX, Lu HF, Zuo J, Sheng JF, Zheng SS, Li LJ (2012) Changes of gut bacteria and immune parameters in liver transplant recipients. *Hepatobiliary and Pancreatic Diseases International* 11:40-50.

Yokota T, Meka CS, Kouro T, Medina KL, Igarashi H, Takahashi M, Oritani K, Funahashi T, Tomiyama Y, Matsuzawa Y, Kincade PW (2003). Adiponectin, a fat cell product, influences the earliest lymphocyte precursors in bone marrow cultures by activation of the cyclooxygenase-prostaglandin pathway in stromal cells. *Journal of Immunology* 171:5091-5099.

Zarkesh-Esfahani H, Pockley G, Metcalfe RA, Bidlingmaier M, Wu Z, Ajami A, Weetman AP, Strasburger CJ, Ross RJ (2001) High-dose leptin activates human leukocytes via receptor expression on monocytes. *Journal of Immunology* 167:4593-4599.

Zinkernagel RM, Pircher HP, Ohashi P, Oehen S, Odermatt B, Mak T, Arnheiter H, Burki K, Hengartner H (1991) T and B cell tolerance and responses to viral antigens in transgenic mice: implications for the pathogenesis of autoimmune versus immunopathological disease. *Immunological Reviews* 122:133-171.