Self-non-self recognition, ischemia, injury, danger, and mobilization of immune defenses: implications for solid organ transplantation.

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INTRODUCTION

Recent data indicate that immune system evolved, not to respond to all "non-self" antigens, but only against "non-self" antigens which injure tissues (1-3). Ordinarily tissue injury occurs during infection and the non-self antigen belongs to the pathogen. By analogy, in transplantation, the tissue injury occurs as an unavoidable result of the surgery and the non-self antigen is the donor major histocompatibility complex (MHC) (4).

In the first half of this Grand Rounds, I will discuss the recognition of non-self renal allografts. In the second half, I will discuss the role of injury in allograft rejection.

PART I: SELF-NON-SELF RECOGNITION IN RENAL TRANSPLANTATION - WHY ARE THERE SO MANY DIFFERENT HLA ALLELES?

A) A "perfect" match between donor and recipient is rare because there are so many different HLA alleles.

For the purposes of clinical renal transplantation, immunologic self is defined by our MHC genes. In humans, these MHC genes are called Human Leukocyte Antigens (HLA) genes which are located at 3 loci. These genes are HLA-A, B, and DR. We receive 3 genes from mother, 3 genes from father, 6 genes altogether. When a transplant is performed the better the matching at these genes between the donor and the recepient, the better the survival of the kidney transplant. See Figure 1.

Clearly, the better the HLA matching, the better the renal allograft survival. Yet, the average match in our Southwest Organ Bank, and in the U.S., is only 2 of 6 possible HLA antigens. Only 3.7% of all transplanted kidneys in the U.S. match at all 6 antigens (5).

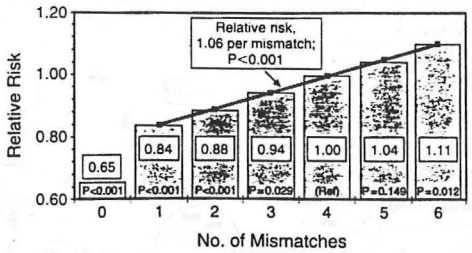

Figure 2. Adjusted Relative Risk of Graft Failure, According to the Number of HLA-A, B, and DR Mismatches among Patients Receiving First Cadaveric Renal Transplants, 1984–1990.

Cox proportional-hazards analysis was used. Other covariates controlled for included those listed in Table 1, except HLA matches. The results from two models are shown: the linear curve presents the number of mismatches from one to six as a continuous variable, whereas the bars present mismatches as six binary variables (with four mismatches as the reference group). Both models were stratified according to transplantation center to control for center effects.

Figure 1: Survival of renal transplants improves with better HLA matching. from Held, P.J. et al. The impact of HLA mismatches on the survival of first cadaveric kidney transplants. *N.Engl.J.Med.* 331:765-770, 1994.

In large part the rarity of a 6 antigen match is due to the extreme polymorphisms of HLA genes. There are 67 known alleles for HLA-A, 149 alleles for HLA-B, and 179 alleles for HLA-DR (6). The polymorphism at these loci is

truely remarkable. If you look at the person to your left and to your right in front of you and behind you it is likely that one of them will have the same hair color or eye color that you have. If we could do transplant typing by hair color or eye color the likelihood of having a good match would be extremely high. In contrast the extreme polymorphism at the major histocompatibility gene loci make the likelihood of having a perfect match extremely small.

The question is why there is such extreme polymorphism at these HLA loci. Clearly, this did not occur solely to frustrate the transplant physician. In order to address this question we need to understand the function of the major histocompatibility antigens.

B) Function of the HLA molecules in the host defense against infection.

A major problem for a host defense system is the fact that many important pathogens live on the inside of our cells. These intracellular pathogens may be divided into two types. One type of pathogens are exogenous. Perhaps the most common clinical exogenous intracellular pathogen is M. Tuberculosis. The macrophage ingests these bacteria, and they then live inside the phagolisosone. How does the immune system know that a particular cell

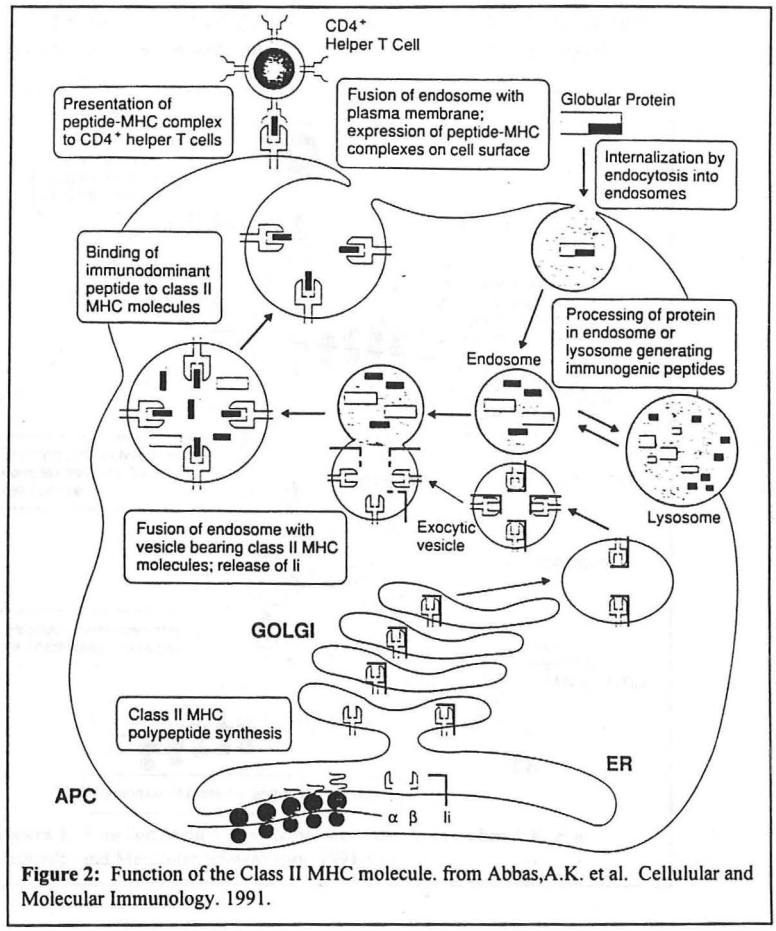

Figure 2: Function of the Class II MHC molecule. from Abbas,A.K. et al. Cellulular and Molecular Immunology. 1991.

has been infected by M-Tuberculosis. The bacteria is on the inside of a cell, the immune system, the T-cells, are on the outside of the cell. The Class II MHC molecules evolved in order to solve this question.

As shown in Figure 2, a macrophage ingests the bacteria which is digested in the phagolysosome. Some of these peptides are transported into another vesicle called an endosone. The Class II MHC enters the endozone and then binds to the peptides of the pathogen. It then shuttles these peptides to the cell surface where they may be recognized by the CD4 helper T cell. In otherwords, Class II major histocompatibility antigen functions as a chaparone which takes peptides from the phagolysosone and shuttles it to the cell surface where the lymphocytes may recognize the foreign antigen and know that an infection is taking place. The Class II MHC evolved to allow the immune system to investigate the contents of the inside of the cell specifically, the intracellular compartment of the phagolysosone. The activated T-Cells secrete various lymphokines which activate the macrophage and cause the bacteria to be destroyed.

Class I MHC evolved to solve a different problem. In some cases the pathogen is a virus. The virus takes over the protein synthetic machinery of the cell. Viral proteins are released into the cytoplasm. The proteasome is a subcellular organelle which ingests these cytoplasmic proteins. The peptides are transported into the endoplasmic reticulum. Within the endoplasmic reticulum the Class I MHC binds to the peptide and then shuttles the peptide to the cell surface where it can be investigated by CD8+ T-cells. The function of the Class I MHC is to allow the immune system to

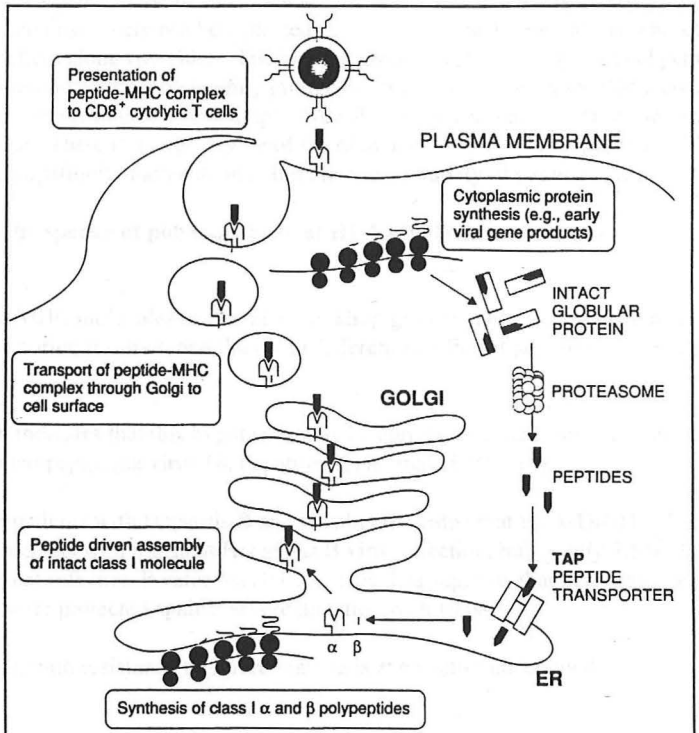

Figure 3: Function of the Class I MHC molecule. from Abbas,A.K. et al. Cellulular and Molecular Immunology. 1991.

investigate the contents of the inside of a cell. In this case, the intracellular compartment is the cytoplasm instead of the phagolysosone (7-11).

Each of us has only 4 types of Class I MHC molecules and two types of Class II major histocompatibility molecules. How is it possible that these 6 molecules have the capacity to bind peptides from all the millions of different kinds of pathogens which infect the insides of our cells? For example, and in contrast, antibody molecules are highly specific and the antibodies which bind to bacteria and viruses on the outside of the cell are specific for viral peptide and will not bind to another type of virus. Yet in order to function properly the six MHC molecules must bind to a diversity of peptides in order to allow the immune system to investigate the status of the inside of our cells. How does this occurr? To answer this question we must look at the struction of the major histocompatibility molecules.

The Class I MHC molecules is a heterdimer consisting of beta 2 microglobulin and a heavy chain, which is divided into 3 domains; $\alpha 1$, $\alpha 2$ and $\alpha 3$. The antigen-binding groove is formed by $\alpha 1$ and the $\alpha 2$ domains. There is a beta pleated sheet forming the bottom of the binding groove and alpha helices along two sides. The binding groove is large enough to bind peptides of approximately 9 amino acids. The binding groove has been constructed such that there are 1, 2, 3 possibly 4 anchoring residues. For example, HLA-B53 requires a proline at the second position o the peptide. There is a large degree of freedom at each of the other positions. Thus, a single major histocompatibility molecule may bind to a large variety of peptides (12).

C) Advantages to the species of polymorphism at HLA - malaria and reverse immunogenetics.

The diversity in the MHC molecules occurs in the binding groove. Different MHC molecules require different anchoring residues, and thus bind different families of peptides. See Figure 4.

There is data which indicates that this hypothesis may be correct for AIDS, cervical cancer associated with human papilloma virus 16, hepatitis B, and malaria (13-19).

An investigation of patients with Hepatitis B and Gambia indicated that HLA-DRB1*1302 was found in 25.6% of patients with transient Hepatitiss B virus infection, but in only 7.5% of patients with persistent infection P value was 0.012. This data suggests that individuals with HLA-DRB1*1302 were protected against severe infection with Hepatitis B.

The HLA association with resistance to severe malaria is even better understood.

Table 1 Structural features and proposed motifs of peptides associated with class I MHC molecules

Isoform	Methods[a]	Predom Length	Side-chain position[b] 1	2	3	4	5	7	C-term	Refs
HLA-A2.1	m, p, s	9		L, M, *I*	phob[c]				V, L, I, A	(30, 39, 71, 72)
HLA-A3.1	e, s	9		L	F				Y, K	(29)
HLA-Aw68	e	9 11		V, *T*					R, K	(26)
HLA-B7	m, p, s	9	A, *R*	P	R, *K*				L, I, *A, V, M*	(32)
HLA-Bw35	p, s	9		P		chg[c]			Y, M, L, I	(73, 74)
HLA-Bw53	p, s	9		P						(73)
HLA-B*2705	e	9	R, *K*	R					K, R	(42)
HLA-A*0205	p	9		V, L, I, Q					L	(41)
HLA-A11	s	9?		L, I					K	(56)
HLA-Bw37	p	8 or 9		D, E			V, *I*	F, M, I, L	(74)	
HLA-B8	p, s	8 or 9			K, R		K, R		L, I	(40)
H-2K[d]	p, s	9		Y					I, L	(39, 75)
H-2K[k]	p, s	8		E					I	(76, 77)
H-2L[d]	e	9		P					F, L	(69)
H-2D[d]	e	9		G	P		R, *K*		I, L, F	(88a)
H-2D[b]	p, s	9					N		M, I	(39)
H-2K[b]	p, s	8			Y, *I*		F, Y, *N*		L, M	(39)
Qa-2[a]	e, p	9		Q, L	L, N			H	L, I, F	(78)[d]
H-2M3	s	?	**N-formyl**							(79, 81)

[a] m, tandem mass spectrometry of individual peptides; e, Edman sequence of individual peptides; p, pool sequence; s, synthetic peptide used to define or confirm a motif.

[b] Single letter amino acid code is used. **Bolded** residues are found in all or most peptide sequences or are considered anchors in pool sequencing. Normal residues are found less frequently at an anchor position, or are at positions considered auxiliary anchors. *Italicized* residues are infrequently found at either position.

[c] phob, hydrophobic residue; chg, charged residue.

[d] Joyce S, Tabaczewski P, Angeletti RH, Nathenson SG, and Stroynowski I, submitted.

Figure 4: Structural features of peptides bound to various MHC molecules. from Engelhard. Annu. Rev. Immunol. 12:181. '94.

The human species has a long interaction with malaria falciparum. There are a number of factors involved in the resistance to falciparum. There are socio economic factors which involve eradication of the mosquito vector as well as the use of mosquito netting, etc. In addition, there are genetic factors. One group of genetic factors involves the red blood cell. This would include sickle-cell trait, α and β thalassaemia, G6PD deficiency, and polymorphism at the TNF alpha promoter (20). An additional genetic factor is the HLA system. The HLA molecule BW53 protects against severe malaria anemia. The incidence of severe malaria in 660 patients was 16.9%, and the incidence of HLABW53 was 22.6% in patients with mild malaria. The incidence of HLABW53 in healthy was 26.4%. This indicates that the presence of HLABW53 confered some protection against severe falciparum malaria.

Understanding the interaction of HLABW53 with malaria may allow development of an effective vaccine against malaria. In the discussion I've already given you HLABW53 should bind to a 9 amino acid peptide from the malaria. It should present this nonapeptide to a T-cell and the T-cell would then eliminate the malaria. Ideally one might construct a vaccine which consisted only of this nonapeptide. Studies of the binding groove of HLABW53 indicate that the peptide requires an anchor residue proline in position 2. The structures of the proteins of malaria falciparum at its various stages have been cloned and sequenced. These include the circumsporozoite protein, the

thrombospondin-related anonymous protein, t he liver-stage-specific antigen-1 or LS1 and the sporozoite hepatocyte-binding antigen. From the known sequences of these 3 proteins one can construct all 60 of the possible peptides with prolene at the second position. One can then make all of these nonapeptides and ask which ones bind HLABW53. It turns out that only the eight peptides bind. One can then take T-cells from individuals with HLABW53 who have mounted a successful immune response against malaria and find out which peptide is recognized by the T-call. It turns out that T-cells recognize the peptide LS6. Thus, one can reconstruct the story with falciparum malaria. In the course of infection with falciparum malaria particularly in the liver-stage, the protiozone degrades the liver-specific antigen into the peptide LS6. Individuals with BW53 are able to bind to this peptide, bring to the cell surface and stimulate T-cells. Individuals with another common HLA type in Gambia HLAB35 have an MHC which is incapable of binding this peptide and therefore not able to present it to its cell surface. This process of determining immunogenic peptide of a pathogen is called "reverse immunogenetics."

The point I have tried so make so far is that MHC Class I and Class II have an important role in allowing the immune system to investigate pathogens living on the inside of cells. We have discussed malaria falciparum in some detail. Diversity in Class I MHC would allow individuals within a population to have greater resistance to some pathogens.

Before leaving this topic I would like to discuss one other illustration of this principle (21). Approximately 56 million people died as a result of the European exploration of the new world. Some of these individuals died in combat, many died as a result of social disruption. However, most of the native Americans died of diseases introduced by the Europeans. It is possible that this occurred because the American Indians did not have HLA types appropriate for defending themselves against diseases transported to the new world by Europeans. Immunologists have taken advantage of the anthropological studies of isolated populations of native Americans in South American and North America. The HLA genes from these populations have been cloned and sequenced. From these studies it is possible to reconstruct the evolution of HLA A,B and C as the native Americans migrated across the Bering Strait into what is now Alaska and then on to South America. It is possible to propose a small group of founding allelas at the 3 Class I MHC sites and then to trace the evolution of new alleles (6).

The anthropological studies demonstrate that the small isolated trial groups in South America have a restricted number of HLA genes within that tribal group. There are an average of only ten alleles at each of the HLA sites. It seems that these tribal groups have developed HLA molecules suited to their survival with those particular pathogens in their particular environment. One might speculate that when the Europeans arrived they brought new pathogens and the HLA antigens of the American Indians were unable to bind to these antigenic peptides, bring them to the cell surface and allow T-cells to become activated.

In the course of this first section of my talk we have discussed the first principle of transplant immunology. That is, self non-self recognition. We have discussed the high level of polymorphism at the major histocompatibility genes and how this complicates renal

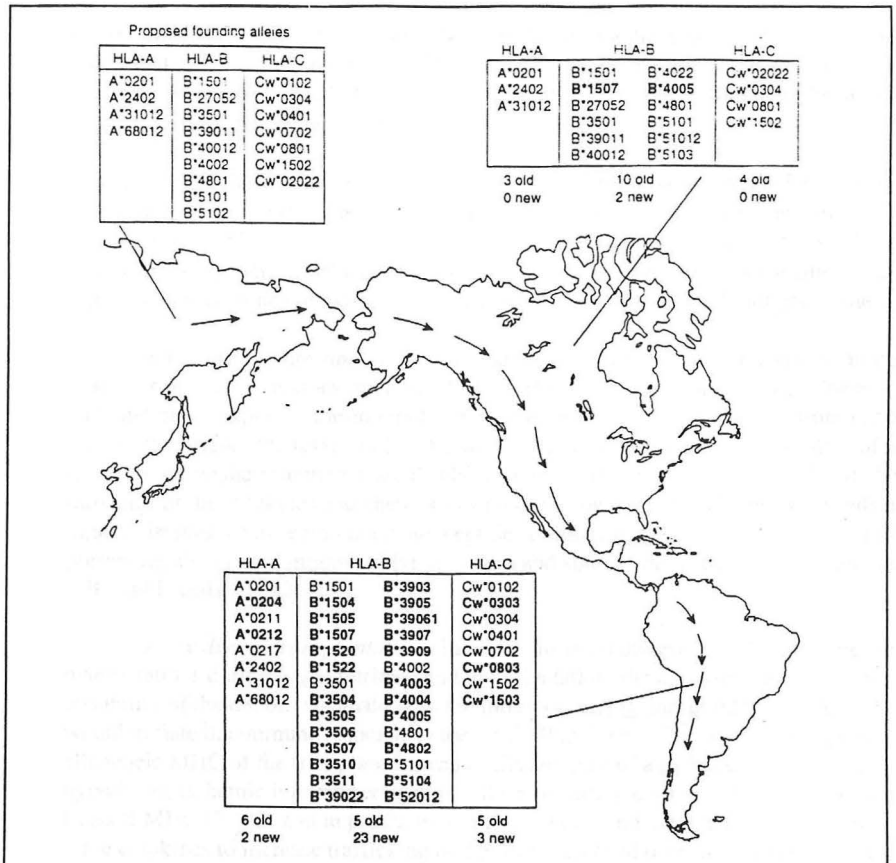

Figure 5: Evolution of HLA genes in Native Americans. from Parham. Science 272: 67 '96.

transplantation. We have discussed why there is so much polymorphism. And in understanding why there is so much polymorphism we have had to understand the function of the major histocompatibility gene.

PART II: Role of ischemia and injury in initiating the immune response. Potential importance in kidney transplantation.

A) Hypothesis.

Althought one important component of allograft rejection is recognition of the non self major histocompatibility antigen, another extremely important component is response of the tissue to

injury. Indeed the point that I would like to make during the next 20-30 minutes is that ischemic injury makes the kidney rejectable. The greater the ischemic injury the greater the rejection. In this sense the transplanted kidney is like or in analagous to tissue which has been injured during the course of an infectious disease.

Recent data emphasize the importance of tissue injury in initiating immune responses (1-3). The idea is that the immune system evolved, not to respond to all "non-self" antigens, but only against "non-self" antigens which injure tissues. Ordinarily tissue injury occurs during infection and the non-self antigen belongs to the pathogen. By analogy, in transplantation, the tissue injury occurs as an unavoidable result of the surgery and the non-self antigen is the donor MHC.

Injury during infection. Infectious pathogens injure tissues. In response to this injury, host cells produce cytokines, such as GM-CSF and TNFα, and chemokines. These activate the endothelium to express adhesion molecules necessary to recruit leukocytes from the blood into the infected tissue (see reviews (22-24)), and direct leukocyte chemotaxis to sites of infection (25). Of particular importance are dendritic cells in this response to injury (3,26). Under the influence of the cytokines and chemokines produced by injured cells, dendritic cells migrate into injured tisssues, capture immunogenic peptides of the pathogen causing injury, acquire antigen presenting ability, and migrate to lymph nodes and spleen where the dendritic cells sensitize T cells and B cells (1-3,26).

Injury during transplantation. Injury to the renal allograft results from the surgical manipulations during organ retrieval and transplantation, the cold storage, and the hemodynamic instability of the donor. By analogy to the injury occurring during infection, the ischemic injury would initiate the immune response to the non-self antigens. The non-self antigens would be the allogeneic MHC of the transplant instead of the antigens of a pathogen. In support of this hypothesis, ischemic injury after clamping the renal artery does result in increased expression of Class II MHC (27,28) and in production of chemokines and cytokines (29,30). One expects these cytokines to increase trafficking of dendritic cells and other leukocytes into the injured kidney.

B) Clues from clinical renal transplantation.

Clinical studies support the above formulation.

The longer the organ preservation time, the greater the relative risk of allograft loss during the first 5 yrs. Figure 6 indicates that injury in associated with organ loss. It does not say that rejection is the cause of organ loss, indeed irreversible injury to the transplant during cold ischemia might be responsible (5). Other data however suggests that rejection is a major factor.

Terisaki has recently reported (31) that patients receiving living-unrelated donor transplants have allograft survival which is much better than patients with cadaveric transplants. This is somewhat surprising since the cadaveric transplants are better matched than the living-unrelated

donor transplants which usually come from the patient's spouse. I suggest that the major difference between the cadaveric and the living-unrelated donor is the fact that the cadaveric kidney is subjected to much greater ischemic injury. The cadaveric kidney suffers injury due to the hemodynamic instability of the donor from the trauma which caused brain death, and also from the prolonged cold ischemia time which is necessary while the receipient is being located and prepared for surgery. On the other hand, a living-unrelated donor transplant is not subjected to hemodynamic instability, and the cold ischemia time is minimal.

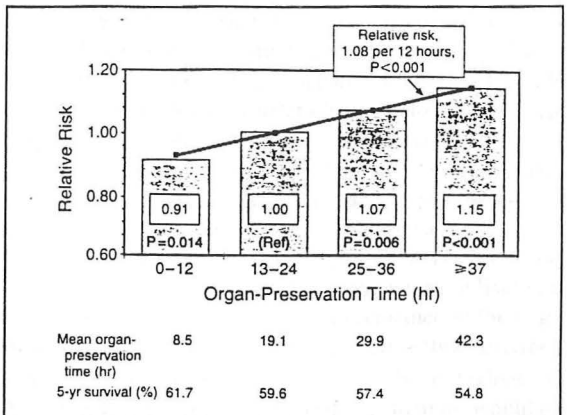

Figure 6: Relative risk of graft failure according to organ-preservation time. from Held. N.Engl.J.Med. 331:765 '94.

An additional clinical series which supports the idea that ischemic kidneys are more rejectable than kidneys suffering less ischemia is the reported by Troppman from The Univ. of Minnesota (32). In this large series from a single center all patients were treated with the identical immunosuppressive regimen. Troppman found that patients who suffered from delayed graft function had a greater incidence of acute allograft rejection than patients who did not suffer from delayed graft function. Delayed graft function is defined as the requirement for one or more dialysis treatment after the transplant. One may think of the allograft with delayed graft function as a kidney with acute renal failure.

C) How would injury initiate rejection?

How might ischemia contribute to rejection? To understand this we might return to the immune response against infections. Afterall, our immune system evolved not to reject transplants but rather to defend the host against infection. Every infection by pathogenic organism causes tissue injury. This tissue injury results in the production of eicosanoids, cytokines and activation of complement. These mediators released by the injured tissue do 3 things: 1) activate the endothelium, 2) recruit dendritic cells into the injured tissue and 3) generate the accessory signals necesary to direct T-cell activation.

One example of this principle that tissue injuries required to generate an active immune response. Our studies done by Dr. Miguel Vazquez when he was working in our laboratory . Miguel studied the reponse of mice injected with listeria which were virulent or listeria which were not verulent. These listeria were genetically engineered such that they differed only in the ability of

the verulent bacteria to express a protein called listeria listeriolysin. Mice injected with listeriolysin positive listeria generated a protective immunity against future large injections of virulent listeria. Mice injected with the non-virulent lisene negative listeria, or killed listeria, did not develop protective immunity. It turns out that only listeriolysin positive listeria damaged tissue. However, after macrophages ingested listeriolysin positive listeria, the listeria respond to the stressfull environment of the phagolysosone by producing their stress protein listeriolysin. The listeriolysin lysis the membrane of the phagolysosone. The bacteria proliferate in the cytoplasm and injure the macrophage. They also reorganize the macrophage cytoskeleton such that the listeria are propelled into a neighboring cell where the entire process is repeated. Miguel's data showed that after macrophages had ingested the lisene negative listeria there was no tissue injury. The endothelium was not activated. Leukocytes remained in the vascular space. On the other hand, if macrophages ingested listeriolysin-positive listeria, these macrophages were injured and the injured cells released interleukin 1, TNFα and other cytokines. These would be expected to activate the endothelium and the activated endothelium would mediate extravasation of lymphocytes, macrophages and polys into the injured tissue. This would generate the immune response. See references (33,34).

The endothelium mediates the leukocyte infiltration into the injured tissue. We now examine this in greater detail. Extravisation of leukocytes might be divided into 3 different stages. A lymphocyte encounters the activated endothelium. It rolls on the endothelium because of low affinity interactions between the polysaccharides on the lymphocyte surface and selectins on the endothelial surface. This rolling slows the leukocyte down such that it can then receive signals from chemotractants generated on the tissue side of the endothelium. These chemokines or chemoattractants cause the leukocytes to change their biology such that they then translocate or extravisate into the injured tissue. On the endothelial side, the activated endothelium rapidly placed P-selectin on its cell surface. P-selectin is preformed within Weidel-Palade bodies. After a time required for new protein synthesis E-selectin is also expressed on an endothelial surface. These selectines bind with their counterligands on the leukocyte cell surface and cause rolling. The rolling leukocyte then may receive signals from chemokines generated on the tissue side of the endothelium. These include MCP1 (or macrophage chemotactic protein 1) Interleukin 8, etc. The leukocyte slows down further and then makes further interactions with the endothelium. The activated endothelium expresses Vcam 1, Icam 1, and Madcam. These are the counter ligands for integrines on the leukocyte cell surface. See review (34) and Figure 7.

The second result of tissue injury is the secretion of mediators which caused dendritic cells to travel to the blood translocate across the endothelium, pick up the antigen and then move to the lymph node. Thus, injured tissues secrete MCSF, GMCSF and IL1. These cytokines are released into the bloodstream, travel to the bone marrow, which generates more leukocytes. The leukocytes go to the blood and they enteract with endothelium which has been activated by these same mediators. The dendritic cells translocate across the endothelium and interact with these same mediators to become differentiated, phagocytose the peptides of the pathogen and then translocate back into the blood and lymph and then on into the lymph node. In order words the dendritic cells are a information system which brings antigen from the injured tissue into the

lymph node. See review (26).

It is currently thought that naive T-cells which have never previously been sensitized by antigen cirrculate between the blood and the lymph node. These naive T-cells are unable to enter extralymphoid tissues such as the kidney or any other tissue which is infected. The naive T-cells encounter dendritic cells in the lymph node. The naive T-cells apparently require the specialized micro environment of the lymph node to become activated. After becoming activated and sensitized these sensitized T-cells migrate through the blood and have the adhesion molecules necessary to travel into extralymphoid tissues. See review (35).

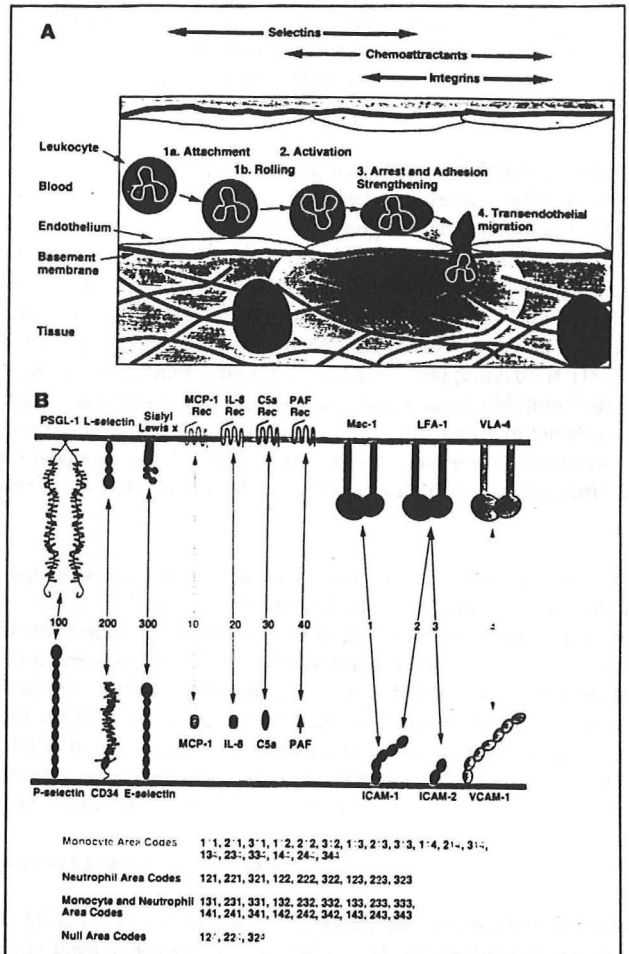

Figure 7: Interactions between endothelium and leukocytes. from Springer. Cell 76:301. '94.

The third result of tissue injury is the generation of accessory signals. T-cells interacting with antigen bound to the antigen binding groove for major histocompatibility antigens are not sufficiently signalled to become activated. These T-cells require additional signals generated by the injured tissue, such as Interleukin 12, B7 in order to form an active response. The nature and the intensity of these accessory signals direct the T-cells down specific pathways, TH1, TH2 and dictate which lymphokines are secreted by the T-cells. See review (36).

Recent data indicate that ischemic injury activates complement which then causes further injury. See reviews (37,38). Injury is ameliorated by inhibition of complement (discussed below).

Examples include skeletal muscle ischemia, intestinal ischemia, liver ischemia, and myocardial ischemia/ infarction. The evidence for complement activation includes increased plasma concentrations of C5b-9 during reperfusion of the ischemic tissue, and deposition of activated complement components in ischemic tissue.

Ischemia activates complement in the following ways (38). 1) C5 is activated by H_2O_2 formed during the reperfusion phase of ischemic injury. Ischemia results in the accumulation of hypoxanthine and other molecules which form toxic oxygen products, including H_2O_2, when oxygen is brought to the cells by reperfusion (39). 2) Ischemia removes inhibitors of complement which ordinarily prevent inappropriate activation of the alternative pathway of complement. In other words, complement is constantly being activated by "C3 tickover", but deleterious effects are prevented by inhibitors on mammalian, but not bacterial, cell surfaces. Two such inhibitors, Decay Accelerating Factor (DAF or CD55) and protectin (CD59) (40), are anchored to the cell surfaces via linkages to membrane glycosyl phosphatidylinositols. Ischemia/ reperfusion activates phospholipase C (39) which cleaves these linkages . In the absence of these membrane inhibitors "C3 tickover" would result in deleterious complement activation. 3) Mitochondrial membranes released by dead or dying cells activate C1q directly, without antibody.

Activation of complement exacerbates ischemic injury (38). The membrane attack complex (C5b-C9) directly damages cells. Furthermore, C5b-C9 stimulates cells which are not irreversibly damaged; for example, C5b-9 stimulates endothelial cells to express E-selectin and ICAM-1 (41). These molecules participate in the extravasation of leukocytes, particularly polymorphonuclear leukocytes (PMN), into the injured tissue. These leukocytes further damage tissue by their secretion of superoxides. C5a also recruits and activates polymorphonuclear leukocytes. The role of PMN in tissue injury may depend upon the length of ischemia. After prolonged ischemia, there may be so much hypoxic tissue damage that there is no further damage when PMN enter the tissue during the reperfusion phase (42).

D) Injury and rejection in rat renal transplantation.

The above discussion has been somewhat theoretical. I would like now to show you some experimental data which has been done by Drs. Stan Sicher and Jeff Penfield in our laboratory using rat kidney transplants and then to also show you some clinical studies which make it likely that these ideas have some merit.

The hypothesis is that all transplanted kidneys suffer some unavoidable injury during procurement of the organ from the donor and during transplant surgery. Allografts from cadaveric donors suffer additional injury from both the hemodynamic instability associated with the acute illness or trauma which caused brain death of the donor, and the cold storage of the kidney while in transit to the recipient. The renal response to this ischemic injury includes inflammation; cytokine/ chemokine production; and increased expression of adhesion, costimulatory, and Major Histocompatibility Complex **(MHC)** molecules on endothelia and

renal tubule cells (RTC) which are the potential targets of alloreactivity (43). We propose that this renal response to ischemia initiates and/or exacerbates renal allograft rejection.

We found that renal tubule cells subjected to ischemia *in vitro* do express increased amounts of mRNA for the chemokine MPC1. This is a molecule which should participate in the signal sequence which causes leukocytes to extravasate into the injured kidney. See Figure 8.

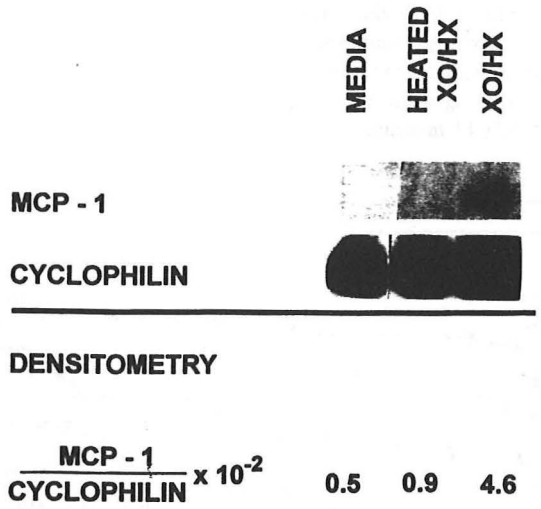

Figure 8: Increase mRNA abundance for MCP-1, a known chemoattractant for dendritic cells. Murine renal tubule cells exposed for 4 hr to the following: left lane, media; middle lane heated xanthine oxidase (XO) + hypoxanthine (HX); right lane, XO + HX which produce superoxide. Top panel: Northern blot probed with cDNA for MCP-1. Bottom panel: densitometry.

Drs. Dawidson, Jim Sentementes, and Amir Rajiv in the Dept. of Surgery, performed a series of kidney transplants for us between syngeneic Wister-Furth rats. These kidneys are not rejected because they are genetically identical rats. These transplants allow the study of renal response to ischemia in a system where there is no alloreactivity. We found that on day 5 there are increased numbers of dendritic cells in the kidney. In addition, on day 5 there is increased amount of ICAM1, an adhesion molecule, on the cell surfaces of renal tubule cells. By 24 hrs. after transplant there is expression of ICAM1 an adhesion molecule on venules and on capillaries. These adhesion molecules should participate in the extravisation of leukocytes into the injured kidney. By day 5 there is also upregulation of ICAM1 on renal tubule cells. This should provide accessory signals for T cell activation (44-46) and also make the renal tubule cell more susceptible to lysis by cytotoxic T lymphocytes (47,48).

E) Ischemic injury and rejection in human kidney transplantation.

Thus in the rat ischemic injury does cause tubular damage, there is secretion of cytokines, there is activation of the endothelium and there is inflammation. All these events should exacerbate or initiate rejection. What about the clinical situation? One mediator of ischemic injury is the

production of superoxide which is produced during the reperfusion phase of ischemic injury. See review (49). In a controlled study performed by the Land group in Germany one half of the transplanted kidneys received superoxide of dismutase and the others did not. This slide shows the results of the study. The patients who received kidney transplants treated with superoxide had better survival at 48 months than patients who did not receive superoxide. More impressively, the allograft survival was 74% in patients receiving superoxide dismutase vs. 52% in patients who did not receive superoxide dismutase. Even more impressive, the number of kidneys which had severe acute rejection in the superoxide dismutase group was 18.5 %, whereas it was much higher in the group not receiving superoxide dismutase at 33.3%. See references (50,51). See Figure 9.

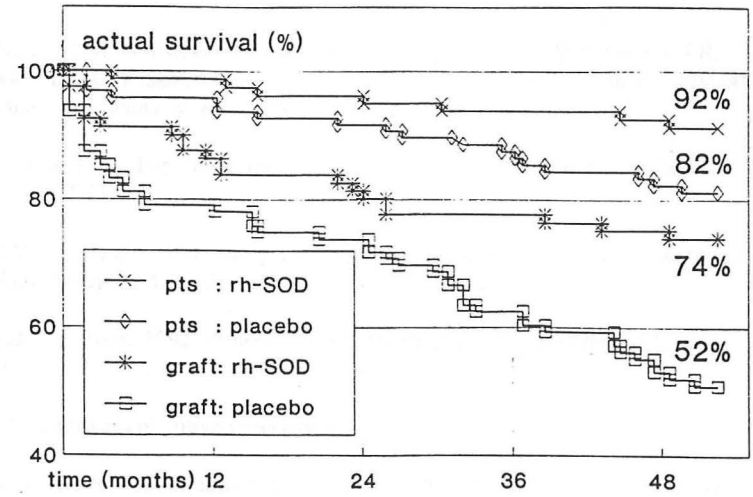

Figure 9: Recombinant human superoxide dismutase prolongs renal allograft survival. from Land. Transplantation 57:211 '94.

An important part of our formulation was that the leukocytes required ICAM1 in order to extravisate into the injured renal transplant and initiate rejection. If this is true then one would expect monoclonal antibodies against ICAM1 to ameliorate rejection. Such a study is in progress. The initial results are extremely hopeful. The patients were selected to be at high risk, i.e., they had a long cold ischemia time. Of the 18 transplants those patients receiving ICAM1 none of them had primary nonfunction. The 16-30 month survival was 38%. In patients receiving the counterlateral kidney and not receiving ICAM1 there was a 30% rate of primary nonfunction and the 16-30 month survival was 56%. The results are not statistically significant at this time because of the small number. See reference (52).

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