SPECIAL ARTICLE
บทความพิเศษ

THE IMMUNE RESPONSE : A TALE OF THREE SIGNALS

Philip F. Halloran, MD, PhD

An understanding of the molecular processes in allograft rejection should be the framework for understanding transplantation and immunosuppression. For those who would understand the scientific basis of the phenomena that occur in clinical transplantation, it is essential to develop a model of the overall function of the immune response ; particularly identifying the essential and non-redundant steps in the immune response ; and to determine how the immune suppressive interventions operate on this response. This will be a dynamic view, which will incorporate new information continuously as it emerges from basic research and clinical experience.

The final test of any of these views is how well it predicts clinical events, and how well it leads to successful applications in the clinic.

The key role in non-specific injury

Injury begets inflammation. In transplantation, a variety of processes lead to at least some degree of injury in the transplanted organ. These include tissue deterioration associated with brain death in the cadaver donor, ischemia associated with harvesting, reperfusion, and the injury associated with rejection itself. Injury increases expression of MHC antigens, and causes infiltration with antigen presenting cells, thus increasing both signal 1 and 2 (see below). Measures of injury in the graft tend to be associated with an increased probability of graft loss in clinical transplantation, particularly graft loss due to rejection. The current hypothesis is that injury leads to rejection which leads to rejection which leads to graft loss. Learning to avoid or to reverse injury may be a key to the better immunologic management of transplants.

The T cell and B cell response

The central event in the immune response to an allograft is the T cell response. The immune response differs from the non-specific inflammatory response in that it is clonal (involving the expansion of small numbers of cells with specific receptors for the antigens of the allograft) ; and that it is adaptive, and displays memory and tolerance. The alloimmune response differs from the more conventional immune response in that there is a much higher frequency of responding cells. Alloantigens may be primarily the peptide presented by the MHC.

The T cell response is a tale of three signals : signal 1 (MHC plus peptide engaging the T cell receptor) ; signal 2 costimulation by ligands on the antigen presenting cell engaging their receptors on the T cell, such as B7-1 and B7-2 engaging CD28 on the T cell ; and signal 3 which is the production of cytokines by the T cell, which engage their specific receptors and deliver the growth (clonal expansion) signal.

The MHC Proteins are Peptide Presenting Structures

The shape of the groove of the MHC protein is affected by polymorphisms in the MHC class I and class II genes. The different shapes of the

grooves for the different alleles influence the peptides which they contain. The strength of the alloresponse between two individuals probably reflects the number of peptides by which they differ. Each individual is tolerant of his or her own peptides, and reactive to the peptide carried in the MHC of other individuals. Other aspects of the MHC molecule other than the peptides that it contains may also evoke responses. In addition, MHC molecule can be broken down into peptides and itself presented in MHC molecules, either of the donor (direct) or the recipient (indirect).

The T cell receptor and CD_4 molecule of the recipient T cell engage the class II molecule and its peptide of the donor on the antigen presenting cell. The T receptor and CD_4 probably crosslink multiple T cell receptor complexes to from multimers or latices. This assembles tyrosine kinases such as Lck, Fyn, and ZAP-70. The activation of the tyrosine kinases phosphorylates the tyrosine kinases themselves, the proteins of the CD_3 complex, and downstream ef fector proteins which begin signalling pathways. Proteins are also attracted to the membrane by their interactions with phosphotyrosine groups, and thus are activated both by tyrosine phosphorylation and by relocation.

Activation of the tyrosine kinases activates several types of proteins which link the T cell receptor to the principal signalling pathways-the calcium-calcineurin pathway and the Ras-MAP kinase pathway.

Tyrosine kinases activate phospholipase C which releases IP3 from the membrane, which in turn releases a transient pulse of calcium from the endoplasmic reticulum. This in turn opens calcium channels in the membrane and causes a sustained rise in intracellular calcium. The high intracytoplasmic calcium activates the phosphatase calcineurin. Calcineurin has several effects on transcription factors. The best known is its ability to activate the cytoplasmic component of the transcription factor NFATp, by cutting many phosphates off serines in a regulatory region of the protein. The NAFTp protein then translocates to the nucleus where it combines with the nuclear component, which may be related to the transcription factor AP-1 (Jun and Fos). This activates NFAT sites in the IL-2 promoter and the promoters of several other cytokines. The process may be regulated by a kinase which phosphorylates and turns off NFATp. For this reason the amount of calcineurin may be rate limiting for all downstream events.

The second major pathway regulating T cell events is the Ras pathway, which is linked to the T cell receptor by linker proteins such as p36, Grb2, and SOS, p21 ras triggers pathways that activate two kinases : the MAP kinases called ERK and the MAP kinases called JNK. An ERK activates a transcription factor called ELK, which activates the C-fos promoter leading to the production of the transcription factor Fos. JNK (Jun N terminal kinase) activates Jun, which together with Fos forms the transcription factor AP-1. Many cytokines and inducible genes have AP-1 sites in their promoters.

Another transcription factor activated in T cells is NF-kB, which activates NF-kB sites. The pathway by which NF-kB is activated in T cells is not clear.

Signal 2 (Co-stimulation)

Several proteins on antigen presenting cells can activate cognate receptors on T cells. The prototype is the activation of the receptor on the T cell called CD28 by the proteins on the antigen presenting cells called B7-1 and B7-2. Other ligand receptor systems on the antigen presenting cell and T cell respectively include CD58-CD2, and CD43 activating its cognate receptor.

CD28 is phosphorylated by a tyrosine kinase, and a protein called PI3-kinase (phosphatidyl inositol 3 hydroxy kinase) is attracted to the phosphotyrosine group in a motif on the CD28. PI3-kinase releases a mediator called PIT-3 (phosphatidyl inositol triphosphate) from the membrane, which activates

protein kinase C isoforms. CD28 activation causes activation of transcription factors AP-1 and NF-kB, which influence cytokines promoters ; and also increases the half-life of cytokine messenger RNA.

CD28 signals and TCR signals may be integrated at the level of Jun kinase (JNK) and cooperate in the activation of Jun and AP-1. Loss of CD28 may lead to a tendency of the TCR signal to generate anergy in the triggered cell.

Signal 3 : Growth Supporting Cytokines Engage Their Receptors

Several cytokines are produced by the activated T cell and engage their receptors on the T cell (autocrine) or adjacdnt T cells (paracrine). Several different cytokines can trigger similar growth signals, activating through some common steps in signal transduction. Thus there is redundancy at the level of the cytokines, but non-redundancy at the level of the signal transduction pathway. For example, the IL-2 knockout mouse is immunocompetent, but micc having knockouts in the IL-2 receptor r chain, which is shared by several cytokine receptors, have severe combined immunodeficiency. This illustrates the principal that some steps in the T cell response are redundant and some are non-redundant.

Certain cytokines trigger the lymphocyte to begin cell division. This process has an obligatory requirement for an intermediate called "target of rapamycin" or TOR. This is a protein with a kinase domain, which probably phosphorylates itself. Rapamycin binds to this and inhibits autophosphorylation. Rapamycin binds as a complex of rapamycin plus FKBP12. This is how rapamycin exerts its immunosuppressive effect, by preventing cytokines from triggering cell division.

The action of TOR probably involves prevention of activation of complexes of cyclins and cyclin-dependent kinases. These enzymes control the transition of the cell from the G1 phase (activation) to the M phase (mitosis). What rapamycin may do is prevent TOR from inactivating an inhibitor called Kip1. In other words, rapamycin inhibits the inhibition of an inhibitor, a very complex mechanism, resulting in the persistence of the inhibitor which prevents the cell from cycling.

When all of the signals are in place, the activated cell begins to cycle. However, for cyling to occur it requires de novo synthesis of purines and pyrimidines. In the absence of de novo synthesis of purines and pyrimidines or in an imbalance between these individual purines (such as an excess of adenine vs. guanine nucleotides) the cell will arrest in the early S phase. This mechanism was first established in hereditary immunodeficiencies. It is also the basis for the use of drugs such as mycophenolic acid (mycophenolate mofetil) in immunosuppression. The drug creates a deficiency of guanine nucleotides relative to adenine nucleotides, by inhibiting the rate limiting enzyme in de novo synthesis of guanine nucleotides, inosine monophosphate dehydrogenase.

Summary

This is order for an immune response to be generated, many requirements must be in place, including antigen engaging the T cell receptor (signal 1), costimuli from the antigen presenting cell engaging their receptors on the T cell, and cytokines produced by the activated T cell engaging their receptors and signalling cell division. The final requirement is for adequate de novo synthesis of purines and pyrimidines.