# *INTRODUCTION*

The vertebrate immune system has evolved to deal with a major problem faced by all higher eukaryotes, namely parasitism by foreign micro-organisms. The general approach to this problem, for the vertebrates, was the development of a recognition system that could both anticipate the antigenic universe and exhibit a high degree of specificity in ligand binding. The key cellular elements of this adaptive immune system are the B cells, T cells and antigen presenting cells (APCs). B cells primarily produce antibodies, and the immunoglobulin (Ig) molecule acts as a soluble receptor which can gain access to the extracellular space, bind the moiety on the free-living extracellular parasite and facilitate its elimination. The binding of the Ig molecule to antigens (Ag) is particularly sensitive to the tertiary structure of the Ags on the micro-organism. Intracellular parasitism posed a more perplexing challenge for higher eukaryotes, which was taken care of by the development of a highly specific cell surface receptor displayed on an effector cell, the T cell. The T-cell receptor (TCR) does not recognize free Ag, but recognizes small peptide fragments (8-15 amino acids long) derived from the proteins of the micro-organisms that are associated with the products of the Major Histocompatibility Complex (MHC) [1], expressed on specialized cells, the APCs. The APCs capture the invading organism, process it and present peptides along with MHC molecules. Peptides are generated from proteins of the invading organism by the action of proteases [2]. MHC-restricted recognition of foreign Ag by the T cells, therefore, reflects the specificity of the TCR for the complex of MHC molecules and Ag fragments and it implies that the TCR must recognize self-MHC

molecules. Education of the T cell repertoire is believed to take place in the thymus, where the T cells that can potentially recognize self-MHC molecules in association with some foreign peptides are allowed to mature, a process called 'positive selection' [7]. On the other hand the auto-reactive cells are deleted, a process known as 'negative selection' [3].

The two major MHC molecules involved in Ag presentation are the MHC class I and MHC class II molecules. These MHC molecules are polymorphic heterodimeric, integral membrane glycoproteins that are encoded by a few loci of the MHC. Both have a membrane anchor supporting a membrane-distal peptide binding groove. This peptide binding groove provides unique specificities for a diverse set of peptides, with variations contributed by allelic polymorphism. There are, however differences between the MHC class I and class II molecules, both structural and functional. Structurally, the peptide-binding groove of the MHC class I molecule is made up of only one polypeptide chain, while two chains contribute to the peptide-binding groove of the MHC class II molecule. Functionally, MHC class I molecules bind to the CD8 co-receptor molecules on the T cells and therefore present Ag to CD8 subset of T cells. MHC class II molecules bind to the CD4 co-receptor molecules on T cells and hence they present Ag to the CD4 subset of T cells. The loading of peptides for MHC class I and class II takes place at different cellular compartments of the APCs.

For peptides to be made available for loading to MHC molecules a complex cell associated machinery of protein degradation is crucial. T cell Ags can be broadly classified into two types, viz., internal Ags and external Ags. Internal Ags are the Ags which are synthesized within the cell and are usually processed through a non-lysosomal route. Peptides derived from these Ags, which are synthesized in the endoplasmic reticulum (ER), associate with the heavy chain of MHC class I and β-2 microglobulin (β2m) in the ER and stabilize this heterodimer. This peptide loaded MHC class I molecule is then expressed on the surface of the cell. Peptides presented by MHC class I includes, in addition to self-peptides, peptides derived from viral proteins and altered self-proteins such as those derived from tumors and thus CD8 T cells mount an immune response against such infections and tumors.

External Ags are the Ags which trace their origin to the exterior of the cell and reach the peripheral endosomal compartments of the cell by endocytic processes. External Ags are processed by the lysosomal route, although some may be processed by surface proteases also, and are presented in conjunction with MHC class II proteins. These include, Ags from extracellular parasites, and some plasma membrane associated proteins, which also reach the endo-lysosomal route because of pinocytosis. MHC class II molecules are intracellularly associated in the ER with a third non-polymorphic 31 kD glycoprotein known as the invariant chain (Ii), which not only prevents binding of any peptide from the ER to the MHC class II molecule, but also targets MHC class II to the endosome region [4,5]. In this region the Ii is cleaved off by the

action of low pH and proteases, after which the MHC class II molecule is loaded with peptides and expressed on the surface. It has been demonstrated, in both the murine and human system, that Ii is not absolutely essential for MHC class II expression [6]. There are instances *in vivo* where MHC class II molecules get to the surface without interacting with Ii, and these molecules have been shown to have a different conformation and may carry a different repertoire of peptides [6].

During thymic education, T cells which recognize self-MHC molecules are selected by a process of 'positive selection'. These positively selected T cells are capable of mounting an immune response against non-self MHC molecules. This ability of T cells to recognize non-self MHC molecules from the same species is called allorecognition [7]. In contrast to the low frequency of T cells specific for conventional Ags, the T cells that are capable of responding to allo-antigen is about 1-10%, which is about $10^3$ to $10^4$ fold higher than the former. The functional consequence of this is the possibility of obtaining a primary T cell response to an allo-antigen, as in an *in vitro* proliferation assay [7]. The models explaining allorecognition fall into two categories, the peptide hypothesis [8] and the density hypothesis [9]. According to the peptide hypothesis, allo-MHC+self-peptide would look like self-MHC+foreign peptide, and thus many foreign-peptide specific T cells would respond and this would increase the frequency of response. The density hypothesis suggests that unlike a nominal Ag, allo-MHC, as a ligand, has a higher density of expression because, T cells have an

intrinsic affinity for allo-MHC, relatively independent of the identity of the peptide loaded. Therefore, APCs presenting allo-MHC will express any where about $10^5$ MHC molecules and this explains the higher frequency of T cells responding to an allo-ligand. Allorecognition has also been demonstrated to be both cell type specific [10] and species-specific [11]. Self-peptides normally associated with MHC molecules are believed to play an important role in allorecognition [7,12]. Since Ii modulates the peptides that bind to MHC class II and its conformation, it may play a role in allorecognition [6].

Recognition of peptide-MHC complex constitutes the primary signal necessary for T cell activation. A complete T cell activation occurs only when a relevant peptide-MHC complex is presented to the appropriate peptide specific, MHC restricted TCR along with other antigen-nonspecific accessory `second' signals by the APCs. In fact there is evidence which says that, if only the `first' signal (peptide-MHC complex) is given in the absence of `second' signals, the T cells are unable to respond to subsequent activation by both `first' and 'second' signal, a state frequently referred to as T cell anergy [13]. The 'first' signal is delivered by cognate interaction between the TCR and its co-receptors, CD4 and CD8, with the peptide-MHC complex . The 'second' signal involves non-cognate interactions between T cells and APCs, through the interaction of cell surface receptor-ligand pairs, such as, CD28-B7/BB1 family of ligands, endothelial leukocyte adhesion molecule (ELAM)-1-E-selectin, very late activation antigen (VLA)-4-vascular cell adhesion molecule (VCAM)-1, lymphocyte function

associated antigen (LFA)-1-intercellular adhesion molecule (ICAM)-1 and heat stable antigen (HSA)-with its ligand [reviewed in 14]. Cytokines such as IL-1 and IL-6 have also been investigated in their role as 'second' signals for T cell activation [15].

A central interaction that has emerged as crucial in these studies is that between CD28 or CTLA4 (Cytotoxic T Lymphocyte Antigen-4) molecules on the T cell and the B7 family of molecules on the APC, and it has been suggested that the provision of this signal in addition to the 'first' signal is adequate to drive optimal T cell stimulation, while its absence during T cell stimulation appears to lead to anergy [reviewed in 16]. CTLA4 is expressed on activated T cells and it has a much greater affinity to B7 family of ligands that CD28.

B cells, macrophages and dendritic cells are the predominant cell types expressing MHC class II molecules and therefore they would normally function as professional APCs for MHC class II-restricted T cell responses [17]. In addition some other cell types, such as, keratinocytes, thyrocytes and human T cells also express MHC class II proteins in certain situations [18] Such non-professional APCs do present Ags, especially peptides, to effector T cells, but the results of such presentation are variable, with T cells proliferation, seen in some instances, while an absence of proliferation and induction of tolerance is seen in others [18,19]. The induction of tolerance could be due to the fact that such APCs may lack one or the other necessary 'second' signals normally provided by professional APCs.

Human T cells express MHC class II upon activation. However, the issue of Ag presentation by human T cells as APCs for CD4 T cell responders is controversial. Some groups have reported proliferation, while others report induction of tolerance in the responder T cells. Most of the work reported on Ag presentation by human T cells to CD4 T cell responders is done using T cell clones as responder [19]. Long-term T cell clones, however, are of the 'memory' phenotype since they have be maintained in culture for many years in some cases. 'Memory' T cells, or 'experienced' T cells are reported to require less stringent stimuli for activation, as compared to naive T cells [20] and, therefore, such studies using T cell clones may not reflect the real significance of T cell APCs *in vivo*. Also, there are some question about how reliably data from long-term T cell clones can be extrapolated to predict the behavior of T cells *in vivo*.

We, therefore, wanted to analyze the Ag presentation and accessory signaling ability of human T cells to function as APCs in the MHC class II context, using freshly purified human T cells as responders. Antigen-specific responses from unprimed cells are normally too small to be detected and hence we have used the allorecognition system, which has higher frequency of primary responses to study T-T interactions. Our results suggest that freshly isolated T cells are capable of 'seeing' alloligands on activated T cells and that such a T-T interaction can induce tolerance in the *ex vivo* responder T cell population [21]. The tolerance induced does not seem

to be influenced by the interaction CD28/CTLA4 and B7 family of ligands or the co-receptor molecule expressed on the APC. These findings have implications both in the mechanistic analysis of T cell activation and in the understanding of T-T interaction.