2 Introduction

Immune responses to microbial heat shock proteins (hsp) are considered a “double edged sword”. On one side, hsp represent prime targets for protective immunity due to their ubiquitous expression among all organisms including microbes. On the other side, the recognition of hsp-antigens from pathogens by adaptive immune receptors is prone to provoke undesirable immune responses to self-antigens due to the high degree of evolutionary conservation of hsp from microbes to mammals. In this thesis an autoimmune response mediated by a T cell clone which reacts with mycobacterial and murine hsp60 antigens is analyzed in detail. Our goal was to study the mechanisms underlying the crossrecognition of self and bacterial antigen with emphasis on the T clone’s individual T cell receptor (TCR).

TCR and B cell receptors (BCR) represent the genes of the adaptive immune receptors. In contrast to innate immunoreceptors, they are organized as large clusters of gene segments that must be assembled during maturation of each immune cell to form an individual clonotypic receptor. Owing to the random nature of the receptor rearrangement, the ligand specificity of these receptors is not predefined and they can thus be reactive towards any self and non-self antigen. Therefore developing T and B cells must undergo selection to ensure tolerance to self-antigens and to avoid autoimmune responses.

2.1 Heat shock proteins

Hsp are constitutively expressed by virtually all eukaryotic and prokaryotic cells, and play various important physiological roles including a molecular chaperone function through the promotion of the folding, assembly, translocation and secretion of newly synthesized polypeptides as well as the participation in the refolding or removal of misfolded or denatured proteins. The name “heat shock proteins” originates from the fact that this group of proteins was discovered through an observation that they are transiently overexpressed after a sudden increase in temperature. Moreover, it was found that hsp expression is generally upregulated as a stress response such as exposure to conditions of cold shock, i.e. to a drop in temperature and various other stresses like radiation, intoxication, anoxia or infection. Hsp subfamilies are defined

by their apparent molecular weights, e.g. hsp100, hsp90, hsp70, hsp60, hsp40 and shsp, small hsp of 15-25 kD. Hsp belong to the most highly conserved proteins in nature regarding structure and function.

Various properties of hsp are relevant for immunity. As it is the case for many other proteins, the correct folding and assembly of immunoreceptors depends on the help of hsp. For instance, the hsp calnexin and calreticulin are essential for the correct folding and assembly of MHC and TCR complexes in the ER (Danilczyk et al., 2000). Furthermore, hsp chaperone and are directly involved in the transport, processing and presentation of antigenic peptides that are generated within cells (Williams and Watts, 1995). Subsequently, peptides that are chaperoned by heat-shock proteins, or are released by cell stress or death, are taken up by antigen-presenting cells (APC) and presented via MHC molecules (Zugel et al., 2001; Li et al., 2002). The receptor CD91 is a common receptor for the hsp gp96, hsp90, hsp70 and calreticulin (Binder et al., 2000b; Basu et al., 2001). Gp96 binding has been shown to induce maturation of professional antigen presenting cells (Singh-Jasuja et al., 2000; Binder et al., 2000a).

Hsp60 seems to be specifically recognized by innate immune receptors on macrophages, and therefore likely acts as a stress or danger signal (Ohashi et al., 2000; Vabulas et al., 2001; Habich et al., 2002; Matzinger, 2002). Because hsp are omnipresent in all living cells and are also upregulated in microbes that are stressed by the host's immune response, they represent excellent targets for the immune response against all kinds of infectious agents (Zugel and Kaufmann, 1999b; Kaufmann, 1990).

In contrast to their immunosupporting activities, hsp are highly suspect as mediators of autoimmunity. Due to their high degree of conservation, an immune response against microbial hsp is likely to result in the propagation of T and B cells that are cross-reacting with self-hsp. This cross-reactivity may be enhanced or triggered by increased expression of self hsp at the site of inflammation (Zugel and Kaufmann, 1999a). In fact, hsp are involved in a variety of human autoimmune diseases (Zugel and Kaufmann, 1999b). αβ T cell responses to mycobacterial hsp60 have been described in rheumatoid arthritis (Li et al., 1992), juvenile chronic arthritis (Graeff-Meeder et al., 1991), Behcet's disease (Pervin et al., 1993) and multiple sclerosis

(MS) (Salvetti et al., 1992). Since hsp are expressed in all tissues, the organ specificity of these autoimmune diseases implies that they are either differentially processed and presented in different tissues, or that other organ specific antigens are primarily responsible for the tissue destruction.

2.2 The innate and the adaptive immune response to hsp

The innate immune system depends on germline encoded receptors that recognize conserved structural patterns of bacterial, viral, parasitic and fungal pathogens (reviewed in (Janeway, Jr. and Medzhitov, 2002)). The discrimination between self and non-self is fail-safe in innate pattern recognition receptors, since they presumably co-evolved with the pathogens. The best studied example of innate immune receptors are the Toll like receptors (TLR), a surface expressed pattern recognition receptor family. The *Drosophila* protein Toll was first described as an important molecule for the dorso-ventral axis formation in fly embryos (Anderson et al., 1985; Hashimoto et al., 1988; Belvin and Anderson, 1996). To date, nine TLR in Drosophila and ten TLR in humans and mice have been described to be involved in the induction of immune response genes (Janeway, Jr. and Medzhitov, 2002). The ligands for each of the different TLR include conserved structures of microbial origin, such as lipopolysaccharide from Gram negative bacteria (Medzhitov et al., 1997), peptidoglycan and Gram-positive bacterial lipoteichoic acid (Takeuchi et al., 1999), unmethylated CpG DNA (Hemmi et al., 2000), flagellin (Hayashi et al., 2001) and the heat shock protein hsp60 (Ohashi et al., 2000; Vabulas et al., 2001). In higher vertebrates the innate immune system serves as a first line of defense, but additionally it instructs and activates the adaptive immune system for the further stages to clear infections. For example, the engagement of TLR on APC results in the expression of the costimulatory molecules CD80 and CD86 which are required for a proper T cell activation (Fearon and Locksley, 1996; Banchereau and Steinman, 1998).

In contrast to innate immunity, adaptive immunity evolved some 450 million years ago, soon after the divergence of jawed and non-jawed vertebrates, when a retrotransposon containing the progenitors of the recombinases RAG-1 and RAG-2 inserted by chance into a DNA sequence encoding a cell surface receptor (Agrawal et

al., 1998; Hiom et al., 1998). As a result, this receptor gene was now separated by the transposon and could therefore only be expressed after recombination of the gene to bring the separated parts together. Multiple duplications and mutations of the separated gene segments created a diverse repertoire of possible receptor rearrangements. As a consequence, each cell that successfully recombined certain receptor segments expressed a particular receptor that differed from the receptors recombined on other cells. This principle of clonotypic receptor recombination is the hallmark of adaptive immunity.

T and B lymphocytes represent the adaptive immune system which enables an organism to mount an immune response to virtually any non-self protein, lipid, carbohydrate or other structure. The adaptive immune system works in coordination with, and uses the same effector mechanisms as, the innate immune system.

2.3 Lymphocytes

B and T lymphocytes are specialized for the generation and utilization of adaptive immune receptors. In most adult vertebrates, the bone marrow harbors hematopoietic stem cells (HSC) which can differentiate into progenitors with more restricted lineage potential and generate the blood cell lineages (**Figure 1**). Self-renewing HSC are multipotent, capable of generating erythroid, myeloid, dendritic, and lymphoid cell lineages. Lymphocyte development occurs through a common lymphoid progenitor (CLP), which has restricted lineage potential (Kondo et al., 1997). CLP undergo B-cell commitment and maturation in response to inductive signals in the bone marrow. CLPs also migrate to the thymus, where they become T-cell-committed.

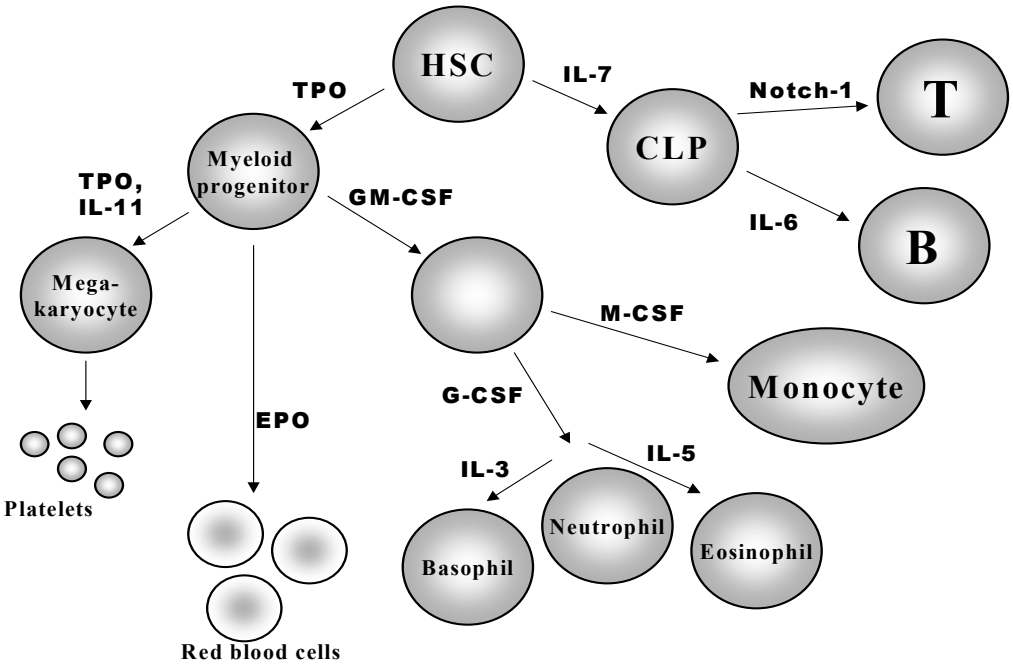

Figure 1. Schematic representation of hematopoiesis in the bone marrow. Regulation of hematopoiesis by cytokines that stimulate the proliferation and/or differentiation of various hematopoietic cells. In the absence of infection, bone-marrow stromal cells are the major source of hematopoietic cytokines. In the presence of infection, cytokines produced by activated macrophages and T-helper cells induce additional hematopoietic activity, resulting in rapid expansion of white blood cells that participate in fighting infection. HSC = hemapoietic stem cell; CLP = commomon lymphoid progenitor; IL = interleukin; CSF = colony-stimulating factor; TPO = thrombopoietin; EPO = erythropoietin. Figure 1 was modified from Kimball's Biology Pages (http://www.ultranet.com/~jkimball/BiologyPages/W/Welcome.html)

B and T lymphocytes employ recombination of the split V (= variable) and J (= joining) gene segments to generate a highly diverse repertoire of antigen specific receptors. In addition, these receptors are expressed as heterodimers of two sequentially rearranged receptor chains which therefore multiplies the potential number of gene recombinations (**Figure 2**). Other processes expand diversity further, such as the D (= diversity) gene segments, the nucleotide-adding enzyme TdT that inserts nucleotides into the V-D-J junctions, and somatic hypermutation of immunoglobulins. Due to gene segment recombination, humans which have approximately 3-4 x 10^5 genes can generate as much as 2.5 x 10^7 different T cell receptors (TCR) and about the same number of B cell receptors (BCR). The ability to recognize virtually every molecular structure allows recognition of non-conserved

microbial proteins, lipids, carbohydrates and toxins as well as tumor specific antigens. The drawback of this enormous receptor diversity is obvious. Because of the random nature of receptor gene segment rearrangement there is no discrimination between self and non-self ligands. The restriction of BCR and TCR to foreign antigens, or in other words the tolerance against self antigens, is controlled by selection of the developing lymphocytes. This selection, however, is not perfect and therefore jawed vertebrates have to deal with the harmful side-effect of the adaptive immune system which is autoimmune disease.

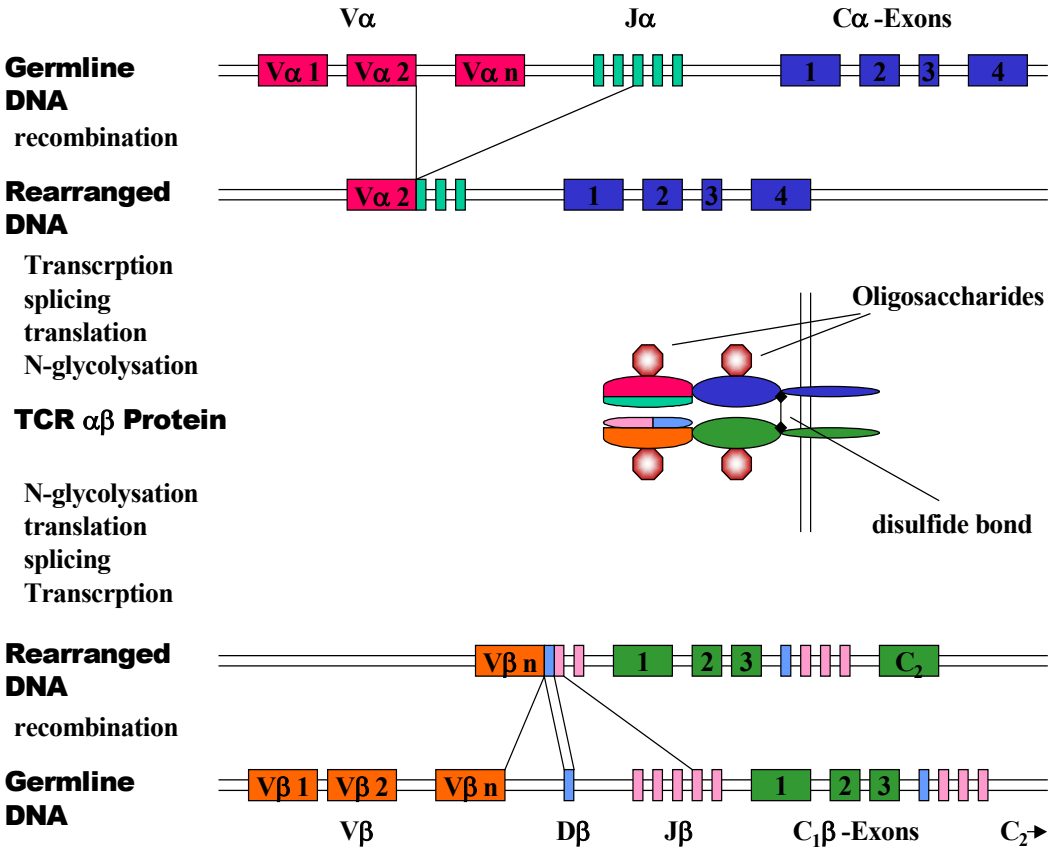

Figure 2. TCR α and β chain rearrangement and expression. The TCR α and β genes are composed of discrete segments that are joined by somatic recombination during maturation of the T cell. For the α chain, a Vα gene segment rearranges to a Jα gene segment to create a functional exon. Transcription and splicing of the VJα exon to Cα generates mRNA that is translated to yield the TCR α protein. For the β chain, like the immunoglobulin heavy chain, the variable domain is encoded in three gene segments, Vβ, Dβ, and Jβ. Rearrangement of these gene segments generates a functional VDJβ exon that is transcribed and spliced to join Cβ; the resulting mRNA is transcribed to yield the TCR β chain. The α and β chains pair to build the TCR αβ heterodimer. The human α chain gene consists of about 70 V = variable segments, and 61 J = joining segments. The β chain gene contains 52 V segments, 2 D = diversity segments, and 13 J segments. Figure 2 modified from (Janeway, Travers et al., 1999).

The present work focuses on T cells which mature in the thymus, the central lymphoid organ specialized to provide an appropriate microenvironment for the maturation and selection of T cells. It is believed that CLP develop into B cells by default (Schatz and Malissen, 2002) but migration to the thymus and signaling of the transmembrane receptor Notch-1 induces T cell lineage commitment (Koch et al., 2001; Wilson et al., 2001). T and B cell development basically follows the same principles. In analogy to B cells, the developmental stages of αβ T cells are marked by the sequential rearrangement of the TCR loci and in addition, the expression of the CD4 and CD8 coreceptors and the cell surface markers CD44 and CD25 (**Figure 3**). The successful recombination of the TCR β chain containing V, D and J segments precedes cell surface expression. Association of this protein with an invariant surrogate α chain (pTα) and CD3 subunits allows signaling events and progression to the CD4 CD8 double positive (DP) stage where TCR β chain rearrangement stops and Vα – Jα rearrangement is induced. Alternatively, CD4 and CD8 double negative T cells may develop into γδ T cells. The factors involved in γδ lineage commitment as well as the physiological role of γδ T cells are presently not fully understood. Upon reaching the DP cell stage, thymocytes initiate TCR α chain rearrangement. Once a functional αβ TCR has formed, DP cells undergo selection. T cells recognize antigen in the form of peptides presented on the cell surface bound to highly polymorphic molecules encoded by the major histocompatibility complex (MHC). DP cells receiving an appropriate signal by intermediate avidity binding of their TCR in concert with either the CD4 or the CD8 coreceptor to MHC molecules are positively selected. In contrast, cells expressing a TCR with high affinity for self-peptide-MHC complexes undergo negative selection by activation induced cell death. Failure to receive any positive TCR-mediated signal results in death by neglect (von Boehmer, 1994; Robey and Fowlkes, 1994; Jameson and Bevan, 1998). Less than 5% of the T cells that mature in the thymus pass the three quality control checkpoints β-selection, positive and negative selection, and leave the thymus as CD4 or CD8 single positive mature T cells.

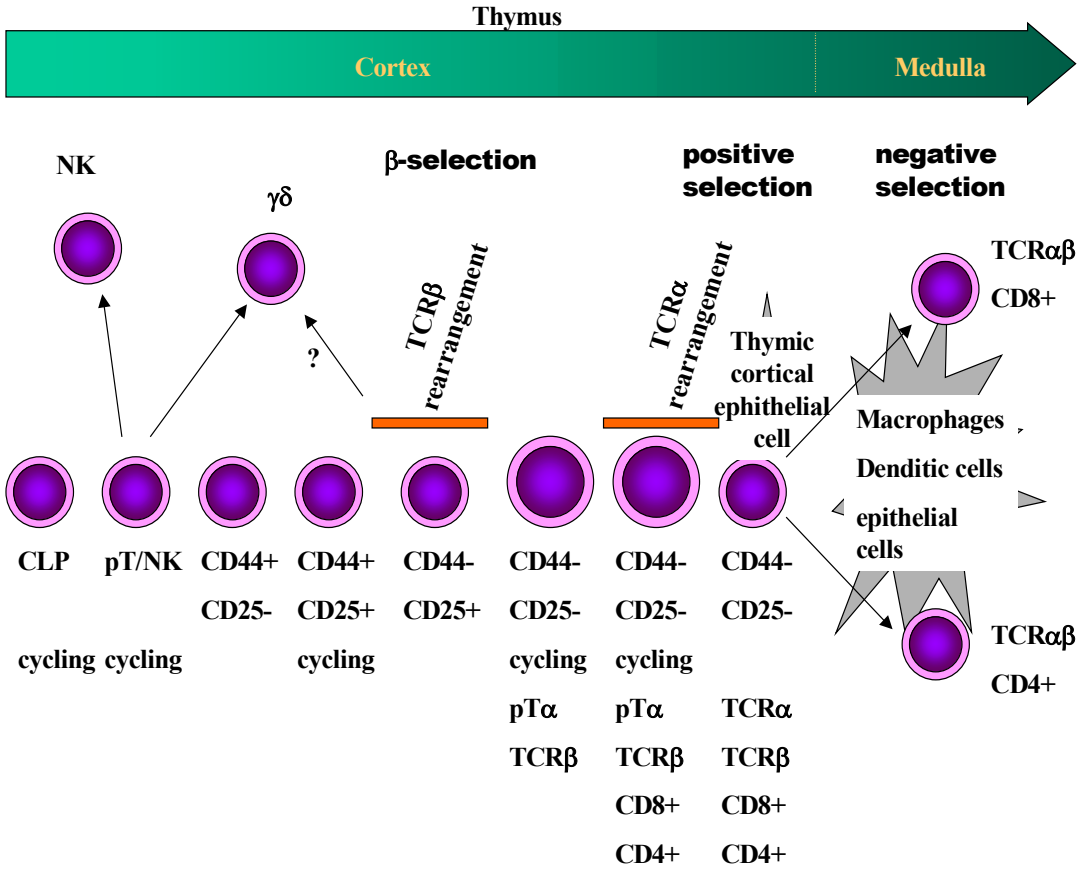

Figure 3. Schematic representation of murine T cell development. Developmental stages of αβ T cells are characterized by the expression of the cell surface markers CD44, CD25, the TCR β chain together with the invariant pTα = pre T α chain, or the αβ TCR, and the TCR coreceptors CD4 and CD8. The stages when TCR rearrangements are occurring are indicated by the horizontal lines. CD4 / CD8 double positive cells need to bind to MHC molecules on thymic cortical ephitelial cells to be positively selected and to progress to the CD4 or CD8 single positive state. High avidity binding of the TCR to self antigens presented by MHC molecules in the medulla results in negative selection of the single positive cells by apoptosis. NK = natural killer cell; CLP = commomon lymphoid progenitor.

The αβ T cells are classified according to the expression of the coreceptors CD4 and CD8 which correlates with the type of MHC molecule that serves as the restriction element: CD4+ cells, which are called T helper cells, are restricted to MHC class II molecules. These molecules are largely confined in their expression to professional APC, i.e. macrophages, dendritic cells and B cells. The length of peptides non-covalently bound into the binding groove of MHC class II molecules is not constrained. Peptides presented to MHC class II usually originate from exogenous protein that is degraded in acidified endocytic vesicles. CD8+ T cells, mainly precursors for cytotoxic T killer cells, are restricted to MHC class I molecules. In

contrast to MHC class II, MHC class I molecules are expressed on all nucleated cells, with the highest expression in hematopoietic cells. MHC class I molecules have a closed binding groove and present short peptides of 8-10 amino acid length. These peptides are mainly derived from posttranslational proteasomal processing and are actively transported by the TAP1-TAP-2 transporter from the cytosol to the ER where they are loaded onto MHC class I. Hence, MHC class I molecules present a set of peptides representative of the proteins present in the cytosol of a cell at a certain time. In case of an infection with intracellular bacteria or viruses, or in the case of mutation, transformation and tumor development, non-self peptides that accumulate in the cytosol will be presented by MHC class I to CD8+ T cells which then can attack and kill the infected or transformed cell.

2.4 T cell receptor signaling

The αβ TCR is expressed in association with an invariant, multimeric CD3 complex comprising a CD3εδ heterodimer, a CD3εγ heterodimer and a CD3ζζ homodimer. The association of the TCR with the CD3 complex controls the surface expression of the TCR αβ chains and the transduction of signals induced by the peptide-MHC complex binding to the variable V domains of the αβ TCR (Lambolez et al., 2002; Hennecke and Wiley, 2001). Since the short cytosolic tails of the TCR αβ chains lack functional signaling domains, the adjacent transmembrane CD3 complex transduces activation signals by interacting with the TCR and various intracellular signaling molecules (Dustin and Chan, 2000). TCR complexes and other signaling components are recruited into sphingolipid/cholesterol enriched microdomains called rafts upon TCR engagement (Xavier et al., 1998; Harder, 2001). The TCR together with the MHC peptide complexes and additional costimulatory molecules form the immunological synapse (**Figure 4**). Subsequent ligand binding of the TCR triggers the activation of receptor associated tyrosine kinases (PTKs) of the Src family such as Lck and Fyn, leading to the phosphorylation of the conserved immunoreceptor tyrosine based activation motifs (ITAMs). Phosphorylation of the ITAMs in the cytoplasmatic tails of CD3 molecules allows binding of SH2 domain containing proteins, such as the cytosolic PTK ZAP-70 which then can phosphorylate the adaptor proteins SLP-76 and LAT. The latter provide a scaffold for the recruitment

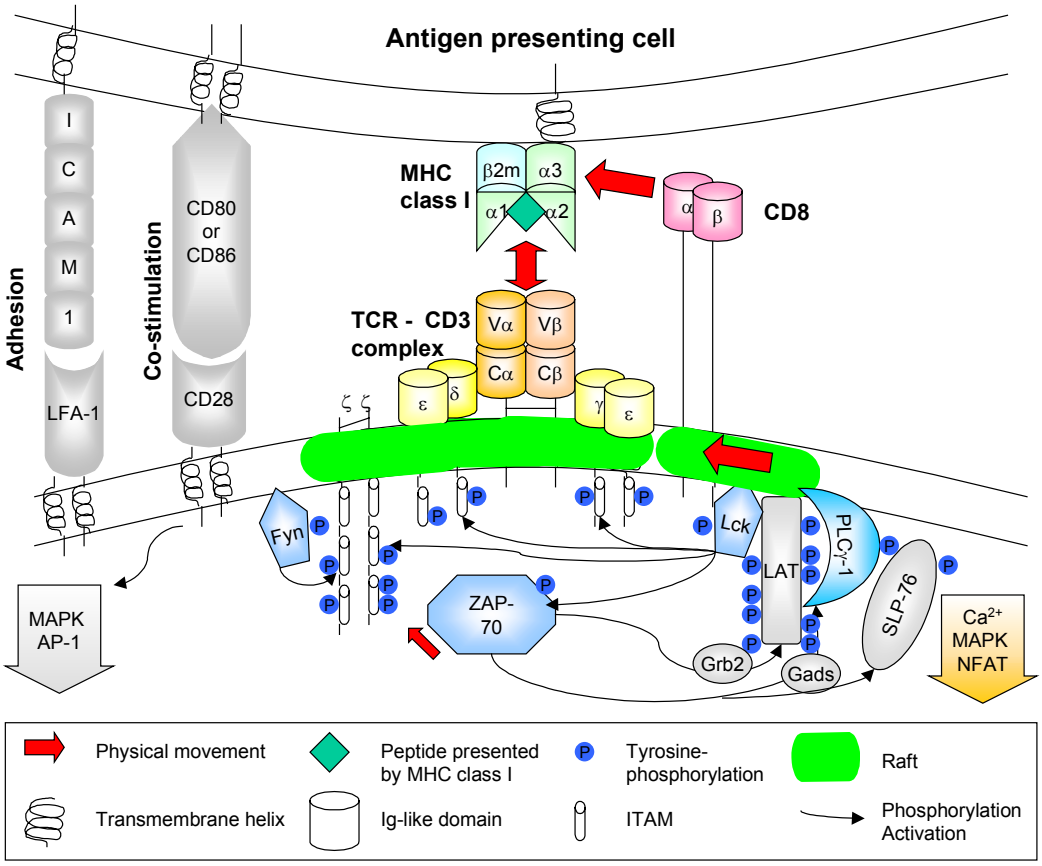

Figure 4. Formation of the immunological synapse between APC and T cell and subsequent signaling events. In the initial encounter of T cells with APC the binding of intercellular adhesion molecules like ICAM-1 to integrins like LFA-1 is crucial. Upon specific recognition of the peptide MHC I complex by the TCR vertical displacement at the TCR and CD3 interface allows association of the intracellular PTK Zap-70 to CD3 ITAMs. Binding of the coreceptor CD8 to the α3 and α2 domains at the lateral site of the MHC class I molecule recruits additional signaling components such as the PTK Lck and the scaffold LAT to the TCR-CD3 raft and completes the signalosome. At this point, CD45 (not shown) dephosphorylates and thereby activates the PTK Lck and Fyn leading to CD3-ITAM and ZAP-70 tyrosine phosphorylation. ZAP-70 then phosphorylates LAT, SLP-76 and PLC-γ1, triggering Ca2+ influx and activation of the transcription factor NFAT. Grb2 and Gads are SH2 domain adaptor proteins. Naive T cells require costimulation via CD28 to CD80/CD86 engagement resulting in activation of the transcription factor AP-1 for complete T cell activation.

of downstream signaling components and control the activation of Ca^{2+} mobilization and MAPK signaling. Depending on the strength and kinetics of the TCR signaling as well as on costimulation, TCR engagement can result in proliferation, activation and expression of effector functions of the T cell, or activation induced T cell death.

2.5 T cell autoimmunity

The adaptive immune system is capable of generating T cells with receptors that recognize virtually any peptide antigen and thus self-reactive T cells are frequently found in healthy individuals. Usually, normal organisms are tolerant to self-antigen, indicating that autoreactive T cells must be inactive. Since lack of T cell tolerance can result in autoimmune pathology, several mechanisms of tolerance induction have evolved, including the following:

Clonal deletion is the physical removal of self-reactive lymphocytes via apoptosis. This occurs mainly at an immature stage of thymocyte development during negative T cell selection in the thymus (Kappler et al., 1987; Kisielow et al., 1988a). A prerequisite for this thymic, central tolerance is the high avidity recognition of self-peptides presented by MHC molecules that are either expressed in or transported to the thymus (Zal et al., 1994; Derbinski et al., 2001). The mechanisms involved in T cell tolerance to proteins that are expressed only in peripheral organs and / or during certain developmental stages are less clear. However, it has been shown that clonal deletion via activation induced T cell apoptosis can also take place in the periphery (Webb et al., 1990; Rocha and von Boehmer, 1991; Schonrich et al., 1994). This mechanism has been termed peripheral deletion.

Clonal ignorance is described by the presence of naive self-specific lymphocytes that are not activated. Clonal ignorance is achieved by the physical separation of specific antigen in tissues from the naive T cells in the blood and lymphatic vessels (Ohashi et al., 1991). Ignorance can be overcome by activated T cells which can invade the tissues and meet their antigen (Wucherpfennig and Strominger, 1995).

Clonal anergy describes a state of T cells that have come in contact with an antigen, but no longer respond to it. The “two signal theory” postulates that in addition to the antigen specific stimulus via the TCR (signal 1), a naive T cell needs proper costimulation (signal 2) to be fully activated (Bretscher, 1975; Bretscher, 1992). The second signal comprises binding of the T cell costimulatory receptor CD28 to it’s ligands, CD80 and CD86, but also signals delivered by soluble factors like cytokines (Schwartz, 1996). TCR engagement without costimulation leads to anergy, a state of functional inactivation.

It is believed that such anergic T cells may play immunoregulatory roles in concert with other subsets of regulatory T cells such as CD4+ CD25+ T cells (Ehl et al., 1998; Ehl et al., 2000; Fowell and Mason, 1993). At present it is not clear whether T cell immunoregulation is antigen specific or if the effects of regulatory T cells are mediated in a broader way via suppressive cytokines like IL-10.

Although there are effective tolerance mechanisms, autoimmune diseases are quite frequent, e.g. at least 5% of the U.S. population are affected (NIH, 2000). Autoreactive CD8+ T cells have been associated with autoimmune diseases including diabetes, rheumatoid arthritis and multiple sclerosis. Both genetic and environmental factors can be involved in breaking immune tolerance. Among the genetic factors, the most important and best characterized susceptibility locus is the MHC, i.e. HLA in humans, H-2 in mice (Vyse and Todd, 1996; Haines et al., 1996). The association of MHC haplotypes with autoimmune diseases underlines the involvement of T cells, whereby the ability of T cells to respond to a particular (auto-) antigen depends on MHC molecules. In most autoimmune diseases, susceptibility is linked most closely to MHC class II alleles. In autoimmune diabetes, however, where both CD4+ and CD8+ T cells are known to mediate the autoimmune response, there are associations with both MHC class I and class II alleles (Ridgway and Fathman, 1998; Gonzalez et al., 1997).

Nevertheless, strong evidence that nongenetically determined factors also influence susceptibility to T cell mediated autoimmune disease is provided by the fact that concordance rates for autoimmune disorders in monozygotic twins range from 25-70%, depending on the disease (Theofilopoulos, 1995). For example, monozygotic twins are usually discordant for type 1 diabetes (Metcalfe et al., 2001; Redondo et al., 2001).

Environmental factors that influence autoimmune disease can be microbes, toxins or dietary products. These factors can activate otherwise ignorant, self-reactive T cells either by inducing antigen-unspecific costimulatory activity, via bystander activation, or via so-called superantigens that directly bind to the TCR complex. Furthermore, continuous stimulation by foreign antigen expressing a high similarity to self, such as shared epitopes of microbial and self origin, may activate previously silent autoreactive T cells (Benoist and Mathis, 2001; Zugel and Kaufmann, 1999b). The

latter mechanism is referred to as molecular mimicry and implies that microbes mimic host components to evade immune recognition. Originally, molecular mimicry was defined through similarities of the antigens at the amino acid level. The concept of epitope mimicry has expanded the definition of molecular mimicry in favor of the hypothesis that structural rather than sequence similarity is the basis of cross-recognition between self and non-self. Several recent studies have demonstrated the degeneracy of the TCR repertoire showing that the sequences of peptides recognized by a single T cell clone express great sequence heterogeneity (Maier et al., 2000; Martin et al., 2001; Gundlach et al., 1996; Hemmer et al., 1998b).

A different type of T cell cross-reactivity that is not based on structural similarities between foreign and self antigens is provided by the naturally occurring expression of two distinct TCR on a single T cell. In contrast to the TCR β loci, where the presence of a productively rearranged β chain prevents further β rearrangements (Uematsu et al., 1988), such allelic exclusion of the TCR α loci is incomplete. Both TCR α alleles rearrange simultaneously and rearrangements continue until a surface TCR αβ receptor is expressed that can be positively selected by thymic MHC molecules (Borgulya et al., 1992). In fact, most peripheral αβ T cells carry VJ rearrangements on both TCR α alleles, and a sizeable fraction of them (40%) show TCR α V–J junctions with a proper translational reading frame on both alleles (Malissen et al., 1992). Therefore mature αβ T cells have the potential to express two TCR α messages and two TCR α proteins (Malissen et al., 1988; Furutani et al., 1989). Various mechanisms of posttranslational allelic exclusion for one of two TCR α chains have been proposed (Alam and Gascoigne, 1998; Sant'Angelo et al., 2001). Estimates of how many peripheral T cells express two distinct surface TCR vary from less than 5% to 30% (Elliott, 1998; Padovan et al., 1993; Elliott and Altmann, 1995; Heath et al., 1995).

In fact, several studies using TCR transgenic mouse models provide evidence that cells expressing two TCR are rescued from thymic deletion and express autoreactive effector functions (Zal et al., 1996; Heath and Miller, 1993; Sarukhan et al., 1998; Hardardottir et al., 1995; Elliott, 1999). Even so, it remains unclear whether dual TCR expression is causally associated with the occurrence of autoimmune disease. Despite the considerable presence of dual TCRs in the normal TCR repertoire, their

role in immune responses to foreign antigen and in the development of self-reactive T cells remains controversial.

2.6 The T cell clone UZ3/4

Mycobacterial hsp are potent antigens that induce T cell responses during mycobacterial infections (Emmrich et al., 1986; Kaufmann et al., 1987). However, owing to the high degree of structural conservation of hsp throughout evolution, crossrecognition of hsp antigens from the host and the invading pathogen poses a potential hazard. Thus, an immune response to hsp has to maintain the balance between protection and damage.

It has been shown that cytotoxic T lymphocytes (CTL) specific for mycobacterial hsp60 peptides are able to lyse stressed target cells (Koga et al., 1989; Munk et al., 1989; Steinhoff et al., 1990). Moreover, it was demonstrated that peptides from endogenous hsp60 served as a target for CTL lysis of the stressed cells (Steinhoff et al., 1994). To further characterize this immune response to self hsp, T cell clones that crossreacted with bacterial and murine hsp60 peptides were established (Schoel et al., 1994; Zugel et al., 1995).

The following work refers to one hsp60 crossreactive CD8+ T cell clone, termed UZ3/4 (Zugel et al., 1995). UZ3/4 was derived from C56BL/6 mice that had been immunized with recombinant mycobacterial hsp60. UZ3/4 lyses *Mycobacterium bovis* BCG infected macrophages, and adoptive transfer of these cells confers some protection against mycobacterial infection (Zugel and Kaufmann, 1997). Furthermore, UZ3/4 cells respond to stressed host cells and therefore are autoreactive. Both the peptide 499-508 of mycobacterial and the peptide 162-170/171 of murine hsp60 are specifically recognized by the CD8+ T cell clone UZ3/4 in the context of H2-D^b MHC class I molecules.

Having shown that mycobacterial infection results in the activation of potentially selfreactive T cells, it was important to test their ability to induce immunopathology *in vivo*. As a first approach, *in vitro* cultured UZ3/4 CD8+ T cells were adoptively transferred into TCR$\beta^{-/-}$ mice lacking endogenous $\alpha\beta$ T cells (Steinhoff et al., 1999). In these mice, the crossreactive T cells induced a severe autoimmune pathology in

the small intestine but not in the colon (**Figure 5**). Similar results were obtained with SCID mice, but not with normal C57BL/6 mice, suggesting that proliferation and effector functions of the cross-reactive UZ3/4 T cells were controlled in immunocompetent mice, probably by other T cells. Taken together, these experiments demonstrate that CD8+ T cells activated during an immune response to mycobacterial hsp are cross-reactive to murine hsp, and cause autoimmune pathology, emphasizing a link between infection and autoimmune disease.

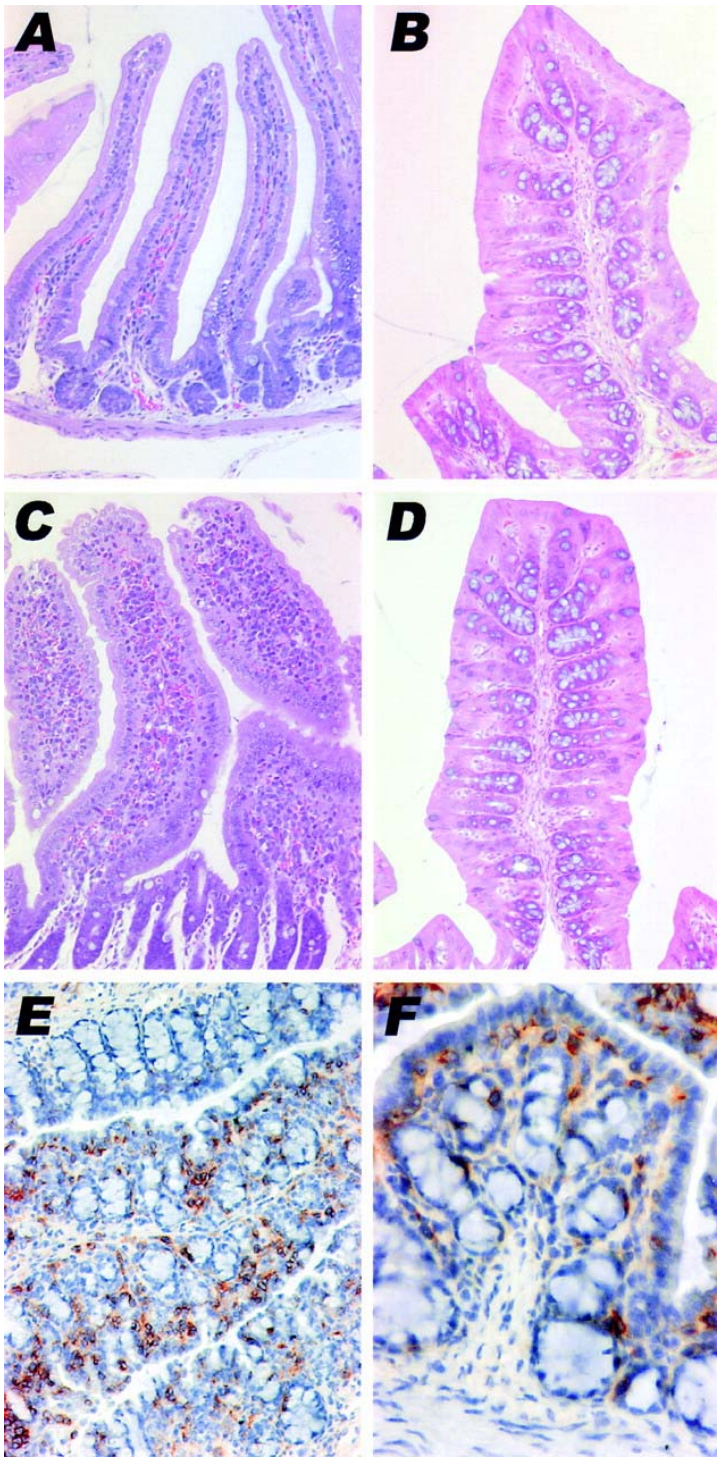

Figure 5. Transfer of HSP60-specific T cells into ß TCR-/- mice leads to inflammation of the small intestine but not the colon. Hematoxylin and eosin stained cross sections of the naive small intestine (A) and the colon (B) and 16 d after reconstitution with HSP60-specific T cells, small intestine (C), colon (D). Cross sections of the small intestine (C) but not the colon (D) show massive expansion of HSP60-specific T cells in the lamina propria and epithelium with degenerative processes at the apical end of the villi. HSP60-specific T cells were detected 16 d after adoptive transfers in the colon by staining for Vß8.1/Vß8.2 positive lymphocytes, overview (E), and detailed section (F). Figure 5 taken from (Kuckelkorn et al., 2002)

2.7 Inflammatory bowel disease

The gastrointestinal tract is the place that contains the highest density of antigens derived from enteric bacteria and ingested nutrients. At the same time it represents an effective barrier to pathogenic microbes. As a consequence, the lymphocytes found in the gut are mainly of an activated phenotype, controlled by a complex, not fully understood network of immunological interactions involving the suppressive cytokines IL-10 and TGF β (Mowat and Viney, 1997). Accordingly, it has been shown in several animal models that disturbance of the tight immunoregulatory network leads to intestinal inflammation.

Inflammatory bowel diseases (IBD) in humans are complex chronic inflammatory disorders of largely unknown etiology (Strober et al., 2002; Wirtz and Neurath, 2000). There are two major forms of IBD, Crohn's disease (CD) and ulcerative colitis (UC). Several mouse models resembling human IBD have recently been developed and have provided new insights into immunoregulatory processes in the gut.

In contrast to the autoimmune intestinal pathology introduced in the previous section, the intestinal antigens involved in IBD induction are not typical self-antigens. They are equivalent to self rather because of their proximity and persistence in the gut. In most of the models dysregulated CD4+ T cell responses to luminal bacterial antigens play a central role in the etiology of the disease (Strober et al., 2002; Iqbal et al., 2002). These CD4+ T cells produce excessive inflammatory cytokines, which leads to infiltration and disruption of the intestinal mucosa by various immune cells. In the large majority of the models the T helper type 1 (Th1) cytokines IL-12, IFN-γ and TNF-α dominate the inflammation, while only a few models are associated with increased expression levels of the T helper type 2 (Th2) cytokines IL-4 and IL-5. There is evidence that CD is associated with a Th1 and UC with a Th2 cytokine pattern of inflammation (Fuss et al., 1996; Parronchi et al., 1997; Takeda et al., 1999).

The most representative model for a Th2 cytokine mediated intestinal inflammation are TCRα $^{-/-}$ mice (Mombaerts et al., 1993). In these mice, pathology resembling UC develops at an age between 4-5 months affecting about 60% of the animals

(Mombaerts et al., 1993; Bhan et al., 2000). An unusual population of Th2 CD4+ TCR$\alpha^{-}\beta^{+}$ T cells is considered to be responsible for IBD induction in TCR$\alpha^{-/-}$ mice (Mizoguchi et al., 1996; Takahashi et al., 1997; Mizoguchi et al., 1997b). These cells infiltrate the large intestine and predominantly produce Th2 cytokines (Mizoguchi et al., 1999). The resulting cytokine imbalance causes proliferation of colonic epithelial crypts and infiltration by large numbers of TCR γδ cells, IgA-producing plasma cells and inflammatory neutrophils into the lamina propria of the colon (Mombaerts et al., 1993). Depletion of CD4+ TCR $\alpha^{-}\beta^{+}$ T cells with mAb directed against the TCR β chain (Takahashi et al., 1997), IL-4 deficiency (Mizoguchi et al., 1999) or IL-4 neutralization (Iijima et al., 1999) inhibit IBD in TCR$\alpha^{-/-}$ mice.

It is generally assumed that the TCR on CD4+ TCR $\alpha^{-}\beta^{+}$ T cells found in TCR$\alpha^{-/-}$ mice is composed of TCR β homodimers. The existence of these TCR β dimers has been shown in the absence of TCR α chains, particularly in TCR β transgenic SCID mice (Kishi et al., 1991), in α -/- mice (Mombaerts et al., 1992; Takahashi et al., 1999), in immature fetal thymocytes (Groettrup and von Boehmer, 1993) and in thymocyte cell lines expressing TCR β only (Groettrup et al., 1992; Kuwabara et al., 1994). The pre-T α receptor (pTα) was found to be absent in CD4+ TCR $\alpha^{-}\beta^{+}$ T cells (Bruno et al., 1995).

2.8 TCR transgenic mice

Usually, the frequency of T cells exhibiting a clonal receptor with a discrete antigen specificity is extremely low. Thus it is difficult to follow such T cell subpopulations *in vivo* through thymic ontogeny and to visualize their fate when released to the periphery. The availability of technology to manipulate the genome of mice by targeting a mutation into a specific gene i.e. knock out mice, or by random integration of an additional gene under the control of a specific promoter into the genome to form transgenic mice, has greatly improved the understanding of the immune system. For the expression of clonotypic rearranged TCR chains the transgenic approach has been proven effective. TCR expression and expression levels are controlled by the regulated expression of the CD3 complex receptor molecules (Biro et al., 1999). Thus, the presence of prearranged TCR chains integrated in the genome of CLP does not influence the development of B cells (which lack CD3 molecules). Also, TCR αβ and γδ lineage commitment seems to be regulated independently of the TCR rearrangement (Terrence et al., 2000).

Complete allelic exclusion of endogenous TCR β rearrangements and partial allelic exclusion of endogenous TCR α rearrangements enriches for T cells with monoclonal expression of the transgenic TCR. However, in all TCR transgenic mice that are principally able to rearrange endogenous TCR chains, this does occur in a proportion of thymocytes which probably suppress the expression of the transgenic TCR. Therefore transgenic TCR expression levels, i.e. the percentage of T cells expressing the transgenic TCR, vary from 0% to greater than 95% depending on the construct used, the integration site, and the copy number of the transgene.

Mice that express a prearranged set of TCR α and TCR β chains in their thymocytes have been invaluable tools for the study of T cell development (Bonneville et al., 1989; Clevers and Owen, 1991), positive and negative T cell selection (Sprent et al., 1988; Sha et al., 1988; Kisielow et al., 1988a; Kisielow et al., 1988b; Ohashi et al., 1990), and the various mechanisms of central or peripheral T cell tolerance (Webb et al., 1990; Ohashi et al., 1991). Furthermore, TCR transgenic mice are very helpful for examination of the mechanisms involved in T cell mediated autoimmune diseases

such as diabetes (Ehl et al., 1997; Suri and Katz, 1999), rheumatoid arthritis (Kouskoff et al., 1996; Matsumoto et al., 1999) and MS (Lafaille et al., 1997).

TCR transgenic mice may be classified in four overlapping groups according to the origin of the antigen recognized by their TCR:

1. TCR specific for model antigens such as ovalbumin (Hogquist et al., 1994), to study basic mechanisms of T cell development, activation and tolerance.
2. TCR specific for self-antigens to study the principles of T cell selection and tolerance, as well as T cell mediated autoimmunity. For example mice expressing a TCR specific for peptides derived from myelin-basic-protein spontaneously develop experimental autoimmune encephalomyelitis (EAE), a demyelinating disease used as a model for multiple sclerosis (Lafaille et al., 1997). Mice expressing a TCR specific for peptides derived from or glucose-6-phosphate-isomerase spontaneously develop rheumatoid arthritis (Kouskoff et al., 1996).
3. TCR specific for microbial antigens to study the T cell immune response to certain pathogens. TCR with specificity for antigens from lymphocytic choriomeningitis virus (LCMV) restricted to MHC class I (Pircher et al., 1989) (Pircher et al., 1990) and MHC class II (Ashton-Rickardt et al., 1994). These mice were used to study the specific T cell response to the LCMV model infection. To analyze the role of antigen-specific CD8(+) T cells for the defense against malaria, T cell receptor transgenic mice specific for the liver stages of the rodent malaria parasite *Plasmodium yoelii* were generated (Sano et al., 2001; Carvalho et al., 2002).
4. Unconventional TCR transgenic mice with unknown antigen specificity. For example, the transgenic expression of a single invariant TCR α chain, V α 14-J α 281, is sufficient information to bias the differentiation of mainstream thymocytes towards the NK1 developmental pathway (Lantz and Bendelac, 1994; Bendelac et al., 1996). These cells appear to be restricted to lipid antigens presented by the non-classical CD1d MHC molecules. Recently, another invariant TCR α chain, Valpha19.1-Jalpha26, was described to facilitate the development of NKT cells which are not CD1d restricted (Shimamura and Huang, 2002).

3 Aims of the study

The goal of this work was to analyze the mechanism underlying the crossreactivity of a hsp60 specific CD8+ T cell clone (UZ3/4). The main focus was on the generation of transgenic mice expressing the TCR of UZ3/4 to study the behavior of hsp specific crossreactive T cells on a clonal level *in vivo*. In contrast to previously described experiments with *in vitro* cultured, already activated T cell clones, TCR transgenic mice allow one to follow thymic selection and to investigate the requirements for activation of naive hsp-specific cross-reactive T cells under physiological conditions. The TCR transgenic mice will be employed to investigate if and how the autoimmune intestinal pathology that was observed by transfer of clonal UZ3/4 T cells can be induced by transgenic hsp60 specific T cells. Activation of these TCR transgenic T cells by mycobacterial infection would provide a link between infection and autoimmune disease.
Furthermore, the TCR transgenic mice that recognize a mycobacterial hsp60 peptide antigen can serve as a model system to monitor specific CD8+ T cell responses to mycobacterial infection. In this context, the TCR transgenic mice may represent a tool to study the function of the CD8+ T cell subset in controlling or eliminating *M. tuberculosis* infections.

Based on our observations that the CD8+ cytotoxic T cell clone UZ3/4 contains two successfully rearranged TCR α chains, specific aims of this work were:

1.) To test the hypothesis that a “dual TCR” is expressed in the T cell clone UZ3/4 and to analyze the involvement of a “dual TCR” in the induction of autoimmune pathology.
2.) To investigate the ability of each TCR α chain to establish a single surface expressed TCR.
3.) To clarify the role of each single TCR with respect to specific peptide antigen recognition.
4.) To evaluate the contribution of both TCR α chains to positive and negative thymic T cell selection.

To achieve these goals, the TCR β and the two TCR α chains of the CTL clone UZ3/4 were dissected

A) *in vitro* in a series of stable transfected cell lines, and

B) *in vivo* by the generation of various TCR transgenic mice.