T cell Receptor αβ Transgenic Mice in Immunological Research

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1. Introduction to T cell physiology

A hallmark of the immune system is its ability to respond to almost any foreign antigen. This extraordinary capability is due both to a vast number of lymphocytes and the fact that each lymphocyte carries a unique, clonally distributed receptor for antigen.

There are two main classes of lymphocytes: [1)]B lymphocytes, which produce antibodies, and [2)]T lymphocytes, which are cytotoxic or secrete regulatory hormone-like substances called lymphokines.

The receptor for antigen on B lymphocytes is membranebound antibody, this receptor binds native antigen in the extracellular fluid. When a B cell is activated it differentiates into an antibody-secreting cell. The secreted antibody enters the circulation, permeates the tissues and inactivates any antigen it may bind.

T lymphocytes, which is the subject of this review, carry a different kind of receptor in their membrane. The T cell receptor (TCR) is not secreted upon activation. Rather, an antigen-activated T cell develops into a cell with cytotoxic and/or lymphokine-producing effector functions. T lymphocytes carry either an αβ receptor or a γδ receptor. The αβ receptor-bearing T cells are by far the most frequent in most mammals and have been studied in most detail (reviewed in Davis & Björkman 1988). Mice have been made transgenic α and β T cell receptor genes and the purpose of this review is to summarize insights into T cell physiology attained by employment of such mice.

The αβ TCR does not recognize native antigen but only small fragments of antigen bound to the groove of Major Histocompatibility Complex (MHC) molecules. There are two types of MHC molecules, class I molecules and class II molecules. (Björkman *et al.* 1987, Brown *et al.* 1993). Class I molecules are present on all nucleated cells while class II molecules are expressed on a limited number of cell types like B cells, macrophages, dendritic cells and thymic epithelial cells. Because neither class I nor class II MHC molecules are secreted, $\alpha\beta^+$ T lymphocytes only recognize peptide fragments presented to them by MHC molecules of neighboring cells. It is important to note that both class I and class II MHC molecules are polymorphic. Allele-specific amino-acid residues are concentrated in the peptide-binding grooves; for that reason different MHC molecules bind and present a different set of peptides to T cells (Björkman *et al.*, 1987, Brown *et al.* 1993, Rammensee *et al.* 1993, Rudensky *et al.* 1992).

In order for a protein antigen to yield small peptide fragments fitting into the grooves on the membrane-distal parts of the MHC mol-

ecules, the protein has first to be proteolytically degraded. It is by now well established that nonsecretory proteins of a cell are continuously degraded by a cytosolic proteolytic machinery and small peptides generated in this process are actively transported across the membrane of the endoplasmic reticulum (ER). In the ER lumen, 8-10 amino acid long peptides bind to the grooves of nascent MHC class I molecules and the complexes are then transported to the cell membrane for presentation to T cells. In contrast to class I-presented peptides (which are derived from cytosolic proteins), class II-presented peptides (12-25 amino acid long) are mainly derived from proteins present in the vesicles of the secretory pathway; these proteins may either have been produced by the cell itself or have been endocytosed (Monaco 1993, Bijlmakers & Ploegh 1993, Neefjes & Momburg 1993).
Corresponding to the distinction between MHC class I and class II molecules, there are two main types of $\alpha\beta^+$ T cells, namely $CD8^+$ and $CD4^+$ T cells. [CD (an acronym for *c*luster of *d*ifferentiation) antigens are membrane molecules expressed by certain cells but not by others]. The CD8 and the CD4 molecules bind to nonpolymorphic, membrane-proximal parts of class I and class II molecules, respectively (Parham 1992). Therefore, $CD8^+$ T cells generally only recognize peptides presented by class I molecules while $CD4^+$ T cells usually only recognize peptides presented by class II molecules. (Fig. 1) (Swain 1983).
The $CD4^+$ and $CD8^+$ T cells carry the same kind of $\alpha\beta$ TCR. The α and B chains are both transmembrane glycoproteins with sequence homology to immunoglobulins. Each chain has a variable (V) domain and a constant (C) domain. It is the $V\alpha$ and the $V\beta$ domains that together define the specificity of a given TCR. Exactly what $V\alpha$ and $V\beta$ domains a certain T cell will express is randomly determined during the development of that T cell in the thymus. The $V\beta$ domain is encoded by three different gene segments ($V\beta$,$D\beta$,$J\beta$) which juxtapose during T cell development.

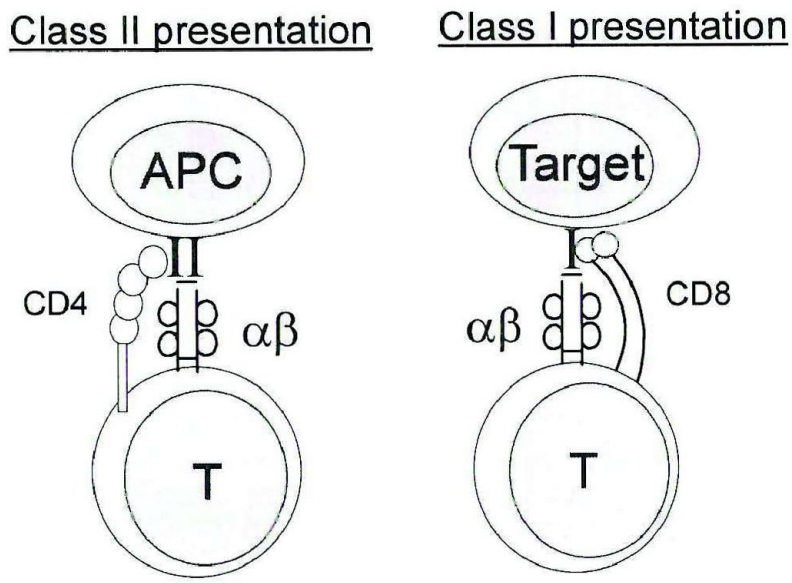

Fig. 1
$CD4^+$ T cells recognize short peptides (–) derived from secretory or endocytosed proteins. The peptide is bound to a groove in the membrane distal part of MHC class II molecules on antigen presenting cells (APC). $CD8^+$ T cells recognize short peptides from cytosolic proteins bound to MHC class I molecules on target cells. For such MHC-restricted recognition of antigen, both types of T cells employ an $\alpha\beta$ T cell receptor.

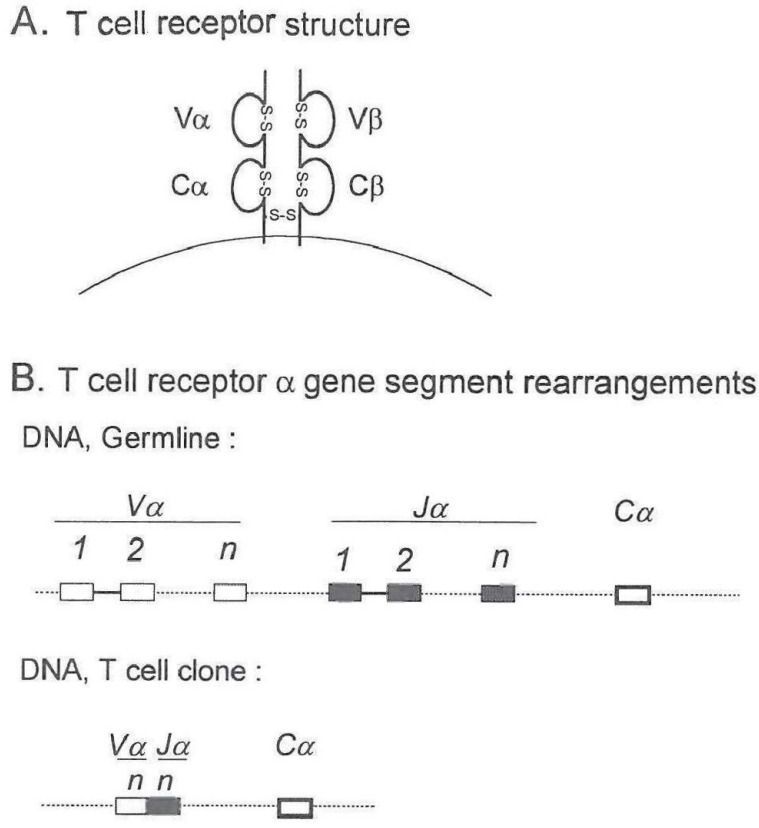

Fig 2.
In A., the structure of the αβ TCR is shown. Ig-like domains are indicated by loops. In B., top part, TCR α gene segments (*Vα, Jα, Cα*) in germline configuration are shown. In a given T cell, a single of the many *Vα* segments (*Vαn*) have rearranged to a single of the many *Jα* gene segments (*Jαn*) (B, bottom part). The *Vα* and *Jα* gene segments will together encode for the Vα region at the protein level. Similarly, the Vβ region is encoded by three rearranged, contiguous *Vβ,Dβ,Jβ* gene segments (reviewed in Davis & Björkman 1988).

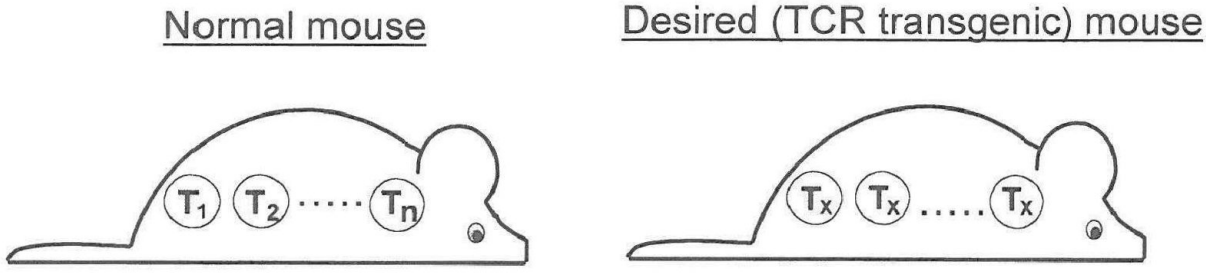

Fig. 3.
In a normal mouse, the vast number of T lymphocytes carry different TCR (T_1, T_2, T_n), making it impossible to follow the physical fate of a T cell with a given TCR. In TCR-transgenic mice, all the T cells generally express only a single TCR (T_x), making it possible to follow their physical fate in bulk during experimental manipulation.

The Vα domain is similarly encoded by Vα and Jα gene segments which join during thymocyte development (Fig.2). There are a large number of gene segments of each class of V, D and J segments, and a major source of TCR diversity is the stochastic combinatorial joining of gene segments during T cell ontogeny. Additional diversity is generated by imprecision of joining (junctional diversity) (Davis and Björkman 1988). Somatic hypermutation, which is a major source of diversity for Ig V-regions, has not been observed for α or β TCR genes. T cells with randomly generated TCR must undergo two types of quality testing before being released for migration to peripheral lymphoid organs. In the positive selection process, T cells with a *weak* affinity for self MHC molecules (presenting self peptides) are selected for survival; teleologically speaking, this is useful because such T cells will in the periphery be capable of employing self MHC molecules for recognition of foreign antigen. (T cells with a TCR having *no* affinity for self MHC die in the thymus because they are useless in the sense that they cannot employ the MHC molecules of the individual for recognition of foreign antigen). In the negative selection process, T cells that recognize self MHC/self peptide complexes with a *high* affinity die by apoptosis. Deletion of such high affinity T cells serves to prevent autoimmunity (Marrack & Kappler 1988, von Boehmer *et al.* 1989).

2. T cell receptor αβ transgenic mice.

A major obstacle in studying T cells *in vivo* has been the large diversity of clonally distributed TCR (see above). It would be much easier to study T cells if the T cells of an animal had identical receptors; then the physical fate of T cells during thymocyte development as well as peripheral immune responses could easily be monitored. Such a desired experimental situation may be obtained by use of mice made transgenic for rearranged TCR α and β chain genes (Fig. 3). The most common and successful way to produce TCR-transgenic mice is outlined in the flow chart of Fig. 4. (Grosveld & Kolias 1992). TCR of both class I and class II-restricted T cell clones have been used to establish T cell receptor transgenic mice. (Table 1,2). Among the class I-restricted receptors, some have been alloreactive to class I molecules, others have had specificity for either a defined antigen or an undefined male antigen dependent on the Y chromosome (Table 1). The class II re-

Table 1. MHC Class-I restricted T cell receptor transgenic strains.

Specificity	*MHC restriction*	*Alloreactivity*	*Reference*
H-Y	D^b		Kisielow *et al.* 1988
LCMV	D^b		Pircher *et al.* 1989
Influenza virus nucleoprotein	D^b		Mamalaki *et al.* 1992
Ovalbumin	K^b		Hogquist *et al.* 1994
SV40 large T	K^k		Geiger *et al.* 1992
		L^d	Sha *et al.* 1988a
		K^b	Schönrich *et al.* 1991
		K^b	Auphan *et al.* 1994

stricted TCR have for the most part been specific for defined peptides (Table 2).

It should be noted (Fig. 4) that the constructs used for microinjection generally is devoid of vector sequences and for that reason their transcription is solely regulated by endogenous T cell receptor gene promoters and enhancers. This strategy ensures that the TCR-transgenes are only transcribed in T cells and not other cells of the body. Since multiple copies of α and β constructs most often co-integrate into the same chromosomal site, it is sufficient that only one of the constructs (e.g. β and not α, Bogen *et al.* 1992) contain an enhancer because that enhancer will also regulate transcription of the enhancer-less construct. In the peripheral lymphoid organs, the TCR-transgenes are usually expressed to a similar level as endogenous TCR-genes in normal T cells, and there is a lack of correlation between the number of integrated transgene copies and TCR expression on the cell surface of peripheral T cells. In the thymus, however, TCR-transgenes are commonly expressed at a higher level early in the ontogeny of transgenic thymocytes, compared to normal thymocytes.

As stated above, the intention of making TCR-transgenic mice is to be able to follow the physical fate of T cells. To be able to do this, it is highly desirable to have a monoclonal antibody (mAb) specific for the heterodimeric αβ TCR encoded by the transgenes. Such clonotypic mAbs (Haskins *et al.* 1983)

Fig. 4. Establishment of ab T cell receptor transgenic mice
1. A T cell of desired antigen specificity and MHC-restriction is selected and α and β TCR genes are cloned (cDNA). Alternatively, PCR is used.
2. Sequence information is used to isolate rearranged genes from a genomic library of the T cell clone.
3. Proper constructs are made containing endogenous enhancers and promoters.
4. α and β constructs, devoid of prokaryotic sequences, are co-injected into fertilized eggs.
5. Offspring are screened for integration by Southern blots or PCR. Expression is characterized by staining with anti-TCR mAbs and Northern blots.
6. A founder is selected and TCR-transgenic mice are bred to obtain desired MHC and genetic background.

Table 2. MHC class II-restricted T cell receptor transgenic strains

Specificity	MHC restriction	Reference
Cytochrome C	$I\text{-}E^k$	Berg *et al.* 1989
Cytochrome C	$I\text{-}E^k$	Kaye *et al.* 1989
Ovalbumin	$I\text{-}A^d$	Murphy *et al.* 1990
$\lambda 2^{315}$ Ig L chain	$I\text{-}E^d$	Bogen *et al.* 1992
Myelin basic protein	$I\text{-}A^u$	Goverman *et al.* 1993
Pancreatic islet cells	$I\text{-}A^{g7}$	Katz *et al.* 1993
Hemagglutinin	$I\text{-}E^d$	Kirberg *et al.* 1994
Myelin basic protein	$I\text{-}A^u$	Lafaille *et al.* 1994
Complement component C5	$I\text{-}E^k$	Zal *et al.* 1994

are often difficult to obtain. If a clonotypic mAb is not available, one may resort to Vα- and Vβ-specific mAb.

A commentary is also needed as to the genetic background of the TCR-transgenic mice (Fig. 4). Immunological experiments often require mice which have a certain MHC haplotype or non-MHC background (e.g BALB/c). If the commonly used F_2 or F_1 eggs are employed for injection (Grosveld & Kolias 1992), establishment of TCR-transgenic mice of a desired genetic make-up often involves a time-consuming breeding schedule. As a shortcut, microinjection may be done into fertilized eggs of the desired strain (e.g. BALB/c), but the rate of success is often far lower than when employing F_1 or F_2 eggs.

It should be noted that investigators generally breed their mice in a heterozygous state (TCR-transgene +/- X -/–) and type offspring for presence of transgenes (50% positive, 50% negative). Even though typing of offspring may be cumbersome, such a breeding scheme results in nontransgenic littermates which may serve as excellent negative controls in most experiments. Another reason for maintaining TCR-transgenic strains in a heterozygous state is that it may prove difficult and time-consuming to obtain homozygous mice, furthermore, such an endeavor may fail altogether because homozygous mice could be nonviable due to a deleterious integration of TCR transgenes.

3. Allelic exclusion in T cell receptor in transgenic mice.

Allelic exclusion, i.e. that only protein from one of the allelic loci is expressed, is know to operate for B cells. TCR-transgenic mice have offered the opportunity to study allelic exclusion of TCR-genes in T lymphocytes. Such studies ask the following question: Does early expression of an already rearranged TCR-transgene suppress rearrangements and expression of endogenous T cell receptor genes? (Note that because the TCR-transgenes generally are integrated outside the TCR loci, their expression will inhibit rearrangement and expression of endogenous TCR-genes on both chromosomes, in *trans*).

In the plurality of instances, a TCRβ transgene appears to rather completely allelically exclude endogenous TCRβ genes (Umeatsu *et al.* 1988). There are, however, exceptions (Bogen *et al.* 1992, Munthe, Sollien & Bogen, unpublished). The allelic exclusion of endogenous TCRα chain genes is less complete (Blüthman *et al.* 1988, Heath & Miller 1993). It is thought that if the transgenic αβ TCR causes efficient positive selection in the thymus, rearrangement of endogenous TCRα genes is fairly effectively prohibited. If, however, the positive selection of the transgenic TCR is marginal, rearrangements of endogenous TCRα chain genes may proceed. The α-chain protein resulting from such an endogenous rearrangement may pair with the transgenic β-chain, resulting in expression of a second receptor which fortuitously can be effectively positively selected by another MHC molecule. Thus, T cells of certain TCR-transgenic strains may indeed express two different receptors. Each of these two receptors will have identical β chains but different α-chains; one which is transgenic and one which is endogenous.

4. Positive selection studied with T cell receptor transgenic mice.

The concept of positive selection, i.e. that thymocytes expressing TCR with *weak* affinities for self MHC molecules are allowed to proceed their differentiation (while other thymocytes die), has been around for more than 15 years (Bevan 1977, Zinkernagel *et al.* 1978). Studies on positive selection have been greatly facilitated by use of TCR-transgenic mice because development of a whole population of thymocytes with identical TCR can be studied. Typically, the studies have been performed by scrutinizing thymocyte development in TCR-TG mice having different MHC haplotypes (Teh *et al.* 1988, Sha *et al* . 1988b, Kisielow *et al.* 1988b, Kaye *et al.* 1989, Berg *et al.* 1989, Pircher *et al.* 1989, Bo-

gen *et al.* 1992). Presence of an MHC haplotype identical to that of the T cell clone from which the transgenes were obtained generally results in enhanced thymocyte survival and differentiation. Even though in most cases not demonstrated, the transgenic TCR is presumably positively selected by the very same MHC molecule that is presenting the ordinary antigenic peptide to the T cell clone which donated the transgenes. In the thymus, the positively selecting MHC molecule is probably presenting various self peptides and *not* the ordinary antigenic peptide for which the TCR is specific. Thus, the transgenic TCR presumably binds weakly to self MHC molecules presenting self peptides (causing positive selection) and strongly to MHC molecules presenting the nominal antigenic peptide.(Hogquist *et al.* 1994, Ashtonrickardt *et al.* 1994).

Positive selection operates on the $CD4^+CD8^+$ stage of thymocyte development and results in enhanced production of single positive ($CD4^+$ or $CD8^+$) mature thymocytes and peripheral T cells expressing the transgenic TCR. If the transgenic TCR is obtained from a class I-restricted $CD8^+$ clone, a lot of $CD8^+$ but few $CD4^+$ mature T cells are generated. Vice versa, if the transgenic TCR is obtained from a class II-restricted, $CD4^+$ T cell clone, plentiful of $CD4^+$ T cells are generated but few $CD8^+$ cells (Teh *et al.* 1988, Sha *et al.* 1988b, Kaye *et al.* 1989, Kisielow *et al.* 1988b, Berg *et al.* 1989, Pircher *et al.* 1989). This phenomenon is called skewing and is probably caused either by instruction or a stochastic process (for review, see Davis & Littman 1994). However, the phenomenon of skewing is not universal and may be dependent on the affinity of the TCR (Bogen *et al.* 1992, Kirberg *et al.* 1994, Bogen *et al.*, unpublished).

5. Negative selection studied with T cell receptor transgenic mice.

T cell tolerance to self antigens is of paramount importance to prevent autoimmunity. A major mechanism of T cell tolerance is deletion of thymocytes with TCR binding with a *high* affinity to self peptides presented by self MHC molecules on dendritic cells or macrophages in the thymus. Studies with TCR-transgenic mice have dramatically demonstrated the physical elimination (by apoptosis) of self-reactive double positive $CD4^+CD8^+$ thymocytes. Such studies have been performed by crossing class I-restricted TCR-TG mice with mice expressing the ordinary antigen in the thymus:

In double-expressing offspring, the thymus is involuted and pronounced deletion of $CD4^+CD8^+$ thymocytes is observed (Kisielow *et al.* 1988a, Sha *et al.* 1988b, Pircher *et al.* 1989). If the class I-restricted, ordinary antigen is not found in the thymus, various forms of peripheral T cell tolerance (deletion, anergy, downregulation of TCR or CD8) is found (Rocha and von Boehmer 1991, Schønrich *et al.* 1991) or the antigen may simply be ignored (Ohashi *et al.* 1991, Miller & Heath 1993). Deletion may also be obtained by injecting antigen into class II-restricted TCR-transgenic mice, in this case the circulatory antigen presumably enters the thymus and is endocytosed, processed and presented by dendritic cells or macrophages (Murphy *et al.* 1990, Bogen *et al.* 1993). In addition to clonal deletion in the thymus, high expression of class-II restricted antigen appears to cause peripheral T cell tolerance by a deletional mechanism (Bogen *et al.* unpublished). The studies referred to above have been performed *in vivo* with TCR-TG mice. In addition, TCR-TG mice is an excellent source of thymocytes with identical TCR to study the mechanism of apoptosis of $CD4^+CD8^+$ cells *in vitro* (Swat *et al.*, 1991, Spain & Berg 1992, Vasquez *et al.* 1992).

6. Peripheral T cell development in T cell receptor transgenic mice.

During the last few years it has become increasingly clear that class II-restricted, $CD4^+$ T cells may secrete different profiles of lymphokines (Mosmann & Coffman 1989). Previously unstimulated $CD4^+$ T cells are called

naive (or virgin) T cells; upon antigenic exposure such cells proliferate extensively and produce IL-2. Subsequent to the primary stimulation, the naive cells differentiate into inflammatory Th1 cells (which produce IFN-γ, TNFβ and IL-2), or helper Th2 cells (which produce IL-4, IL-5, IL-6). To decipher the stimuli which gear the differentiation either towards Th1 or Th2 development is of obvious importance to vaccination and a better understanding of autoimmune and infectious diseases.

T cell receptor transgenic mice offer an excellent opportunity to study Th1/Th2 development. In such mice, most $CD4^+$ T cells will be of a naive phenotype because the receptor for which they are transgenic will not have been stimulated by environmental antigens. [This presumption may not always be true because T cells of TCR-TG mice may occasionally express two receptors; therefore, the T cells may have been primed by their second, nontransgenic TCR. This ambiguity may, however, be removed by studying TCR-TG mice made homozygous for the *scid* mutation (Scott *et al.* 1989, Bogen *et al.* unpublished)]. Since most T cells of TCR-TG mice are of a naive phenotype, such mice may be exposed to various forms of the antigen in the presence of certain lymphokines or anti-lymphokine mAbs, and the subsequent Th1 and Th2 development can be monitored (Hsieh *et al.* 1992, Seder *et al.* 1992, Croft *et al.* 1992).

7. T cell receptor transgenic mice in studies on disease states.

The immunosurveillance hypothesis suggests that lymphocytes scan the body for cancerous cells and eliminate them. The hypothesis implies that cells of a naive phenotype can home to a site of an incipient tumor, become activated and destroy the tumor cells. It has been difficult to substantiate the immunosurveillance hypothesis. TCR-transgenic mice, however, offer an opportunity to evaluate the immunosurveillance hypothesis: A mouse transgenic for a TCR recognizing a tumor-specific antigen can simply be injected with tumor cells and monitored for tumor development.

Such an experiment has recently been performed. A mouse plasmacytoma cell, MOPC315, produces a myeloma protein with a $\lambda 2^{315}$ light chain. The myeloma protein is processed by APC and a peptide comprising residues 91-101 of the $\lambda 2^{315}$ chain is presented by the I-E^d MHC class II molecule (Bogen *et al.* 1986, Weiss & Bogen 1989). The 91-101 peptide contain three unique residues, Phe^{94} Arg^{95} Asn^{96} which are unique to the $\lambda 2^{315}$ chain due to somatic mutations. T cells are specific for these three unique residues. Thus, T cells can recognize an antibody variable region determinant, called an idiotypic peptide, which may be considered a tumor-specific antigen unique to the plasmacytoma cell *in casu*. Mice have been made transgenic for a 91-101 ($\lambda 2^{315}$)-specific TCR (Bogen *et al.*, 1992). Such TCR-TG mice were indeed specifically protected against a challenge with MOPC315 cells (Lauritzen *et al.* 1994). Furthermore, TCR-TG mice made homozygous for the *scid* mutation were also protected, implying that B cells and antibodies were not required for protection. (Bogen *et al.*, unpublished).

TCR-transgenic mice have also been performed to study the role of T cells in eliciting autoimmune diseases. For example, mice transgenic for myelin basic protein-specific TCR develop experimental allergic encephalomyelitis, (Goverman *et al.* 1993, Lafaille *et al.* 1994). In another model for autoimmune disease, mice transgenic for a TCR derived from a diabetogenic T cell clone develop spontaneous diabetes (Katz *et al.* 1993). Studies have also been performed in which a foreign antigen, lymphocytic choriomeningitis virus (LCMV) glycoprotein, was expressed under the influence of the rat insulin promotor on β-islet cells of transgenic mice. Such mice did not develop diabetes even when crossed with mice transgenic for a LCMV - specific, class I- restricted TCR (Ohashi *et al.*, 1991). This indicates that naive T cells ignore LCMV antigen aberrantly expressed in the

pancreas. However, a T cell attack against β-cells, could be elicited by infection of double transgenic mice with LCMV virus, resulting in development of diabetes (Ohashi, 1993). Diabetes was also readily observed in LCMV/TCR/B7 triple transgenic animals (Harlan *et al.*, 1994). The latter two reports indicate that professional antigen-presenting cells, possessing costimulatory activity (B7), are important for activation of autoreactive T cells. Once activated, such T cells can subsequently attack other cells, e.g. the β-islet cells in the pancreas. In conclusion, TCR-transgenic mice hold big promise for elucidating the pathogenesis of autoimmune diseases.

8. Summary

T cell receptor transgenic mice have had an increasingly large impact on immunological research for the last 8 years during which such mice have been available. The mice have been important to further our understanding of T cell receptor acquisition and thymocyte development. In the coming years, it is anticipated that the mice will contribute to our apprehension of peripheral T cell activation and differentiation, and to the role of T cells in disease states.

Sammendrag:
T celle reseptor transgene mus har hatt en økende innflytelse på immunologisk forskning i løpet av de siste 8 årene slike mus har vært tilgjengelige. Musene har vært viktige for vår oppfatning av utvikling av thymocytter og dannelse av T celle reseptor repertoiret. I de kommende år forventes det at T celle reseptor transgene mus vil gi vesentlige bidrag til vår forståelse av perifer T celle aktivering og differensiering, samt T cellers rolle ved sykdomstilstander.

Acknowledgements:
Suzanne Garman-Vik expertly prepared the manuscript. Ludvig Munthe kindly made the figures. The contribution of the various collaborators and co-workers participating in the establishment of the $\lambda 2^{315}$-model are gratefully acknowledged. B.B. is supported by the Norwegian Cancer Society and the Norwegian Research Council.

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