51 Siminovitch, K. A., Bakhshi, A., Goldman, P., and Korsmeyer, S. J., A uniform deleting element mediates the loss of κ genes in human B cells. Nature *316* (1985) 260–262.
52 Snapper, C. M., and Paul, W. E., Interferon-γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science *236* (1987) 944–947.
53 Stall, A., Kroese, F. G., Gadus, F. T., Sieckmann, D. G., Herzenberg, L. A., and Herzenberg, L. A., Rearrangement and expression of endogenous immunoglobulin genes occur in many murine B cells expressing transgenic membrane IgM. Proc. natl Acad. Sci. USA *85* (1988) 3546–3550.
54 Storb, U., Pinkert, C., Arp, B., Engler, P., Gollahon, K., Manz, J., Brady, W., and Brinster, R., Transgenic mice with μ and κ genes encoding antiphosphorylcholine antibodies. J. exp. Med. *164* (1986) 627–641.
55 Storb, U., The published data. Immun. Rev. *115* (1990) 253–257.
56 Suematsu, S., Matsuda, T., Aosaza, K., Akira, S., Nakano, N., Ohno, S., Miyazaki, J., Yamamura, K., Hirano, T., and Kishimoto, T., IgG1 plasmacytosis in interleukin 6 transgenic mice. Proc. natl Acad. Sci. USA *86* (1989) 7547–7551.
57 Tepper, R. I., Levinson, D. A., Stanger, B. Z., Campos Torres, J., Abbas, A. K., and Leder, P., IL-4 induces allergic-like inflammatory disease and alters T cell development in transgenic mice. Cell *62* (1990) 456–467.
58 Tominaga, A., Takaki, S., Koyama, N., Katoh, S., Matsumoto, R., Migita, M., Hitoshi, Y., Hosoya, Y., Yamauchi, S., Kanai, Y., Miyazaki, J., Usuku, G., Yamamura, K., and Takatsu, K., Transgenic mice expressing a B cell growth and differentiation factor gene (Interleukin 5) develop eosinophilia and autoantibody production. J. exp. Med. *173* (1991) 429–437.
59 Tonegawa, S., Somatic generation of antibody diversity. Nature *302* (1983) 575–581.
60 Tsubata, T., and Reth, M., The products of pre-B cell-specific genes (λ5 and V_{preB}) and the immunoglobulin μ chain form a complex that is transported onto the cell surface. J. exp. Med. *172* (1990) 973–976.
61 Warren, D. J., and Moore, M. S., Synergism among interleukin 1, interleukin 3, and interleukin 5 in the production of eosinophils from primitive hemopoietic stem cells. J. Immun. *140* (1988) 94.
62 Weaver, D., Constantini, F., Imanishi-Kari, T., and Baltimore, D., A transgenic immunoglobulin Mu gene prevents rearrangement of endogenous genes. Cell *42* (1985) 117–127.
63 Weaver, D., Reis, M. H., Albanese, C., Constantini, F., Baltimore, D., and Imanishi-Kari, T., Altered repertoire of endogenous immunoglobulin gene expression in transgenic mice containing a rearranged Mu heavy chain gene. Cell *45* (1986) 247–259.
64 Yancopoulos, G. D., and Alt, F. W., Developmentally controlled and tissue-specific expression of unrearranged V_H gene segments. Cell *40* (1985) 271–281.

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Analysis of the immune system with transgenic mice: T cell development

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Abstract. Transgenic mice carrying functionally rearranged T cell receptor genes have contributed significantly to our knowledge of T cell development and thymic positive and negative selection processes. In addition, TCR-transgenic mice have been used to investigate mutations affecting thymocyte development, like *scid* and *lpr*. Gene targeting by homologous recombination will allow to analyze more specifically the molecular mechanisms underlying thymic selection and peripheral tolerance.
Key words. Transgenic mice; immune system; T cell development; T cell receptor; *scid* mutation; *lpr* mutation.

1. Control of T cell receptor (TCR) gene rearrangement in TCR transgenic mice

The enormous diversity of TCR specificities enables the immune system to mount a specific immune response to virtually any given antigen the host may encounter. This diversity is generated by somatic rearrangements of distinct germ line gene segments during T cell development and the addition of N regions[17]. Thymocyte precursors from the bone marrow colonize the thymus at day 14 of gestation[9], are induced to proliferate and start to rearrange their TCR loci. Rearrangement and expression of TCR loci is temporally ordered and lymphocytes expressing γδ or αβ TCRs appear sequentially during thymic development[25]. γδ T cells are found in the thymus and peripheral lymphoid organs at relatively low frequencies and constitute about 5–15% of peripheral T cells. However, they are present more abundantly in certain ephithelia like skin[34, 62] and small intestine[7, 22]. Little is known about their function (for review see ref. 26). They have been implicated in defence against mycobacteria and other infectious organisms and shown to be specific for heat shock proteins (reviewed in ref. 8). Whether γδ T cells are subject to thymic selection processes is still under investigation.

The majority of T cells express an αβTCR. The TCRβ locus is composed of about 30 variable (Vβ) gene segments and two tandemly arranged clusters each coding for 1 diversity (Dβ), 6 functional joining (Jβ), and 1 constant (Cβ) gene segment[14]. The α locus consists of about 50 Vα, 50 Jα, and a single Cα gene segment[72]. Rearrangement starts on the TCRβ locus at about day 15 in gestation[9] by joining a Dβ segment to one of the Jβ elements. In a second step a Vβ region is fused to the DβJβ joint. TCRα rearrangement takes place a few days later and is a one-step process by which a Vα segment is combined directly to one of the Jα regions.

Sequence analysis of TCRβ loci of cloned T cells has revealed that functional rearrangements occur only on one chromosome leaving the other allele non-functional-

ly rearranged[13, 23]. This process, known as allelic exclusion, leads to the expression of only one TCRβ chain on every developing T cell and is a prerequisite for the clonal distribution of different TCR specificities on mature T cells. Initially these results were interpreted in terms of a stochastic model, meaning that the probability for two functional rearrangements is too low to occur on both alleles in a given T cell[2, 15, 51]. Analysis of transgenic mice carrying a functional TCRβ gene, however, has shown that allelic exclusion is a regulated event[67]. Introduction of a productively rearranged TCRβ gene into the germ line of transgenic mice completely suppressed endogenous rearrangements at the V to DJ, but not at the D to J level. Suppression of endogenous genes was found to depend on the amount of transgenic β chain expressed. In mice where the surface expression of the transgene was low, both transgenic and endogenous TCRβ chains were detected on the surface of the same cell[53]. In normal T cells, violation of allelic exclusion of the TCRβ locus was reported in two cases so far[43, 56]. An accessibility model was proposed[73] to account for the control of Ig and TCR variable region gene rearrangements in lymphocytes. In this model stage-, tissue-, and allele-specific V gene assembly is controlled by modulating the accessibility of the loci to a common VDJ recombinase through changes of their chromatin structure.

Whereas allelic exclusion seemed to be rather complete for the TCRβ locus, it was found to be less strictly controlled for TCRα genes. Several T cell clones were isolated with two productively rearranged α chains.[19, 21, 39, 40]. Moreover, in $\alpha\beta$TCR transgenic mice rearrangements were found to occur on endogenous TCRα loci[6]. It seems that functional rearrangement and expression of one allele is not sufficient to shut off VDJ recombinase activity. The results also indicate that a clonally distributed TCR repertoire might not be controlled exclusively at the level of gene rearrangement.

Transgenic mouse models have been designed to study the requirements for allelic exclusion. Krimpenfort et al.[33] used as a transgene a functional TCRβ gene from which the V region was deleted, leaving the remainder including the promotor and the leader sequence of the V region intact. They found that also the mutated TCRβ gene imposed allelic exclusion on the endogenous loci. This inhibition was mediated by the truncated TCRβ protein as no allelic exclusion was found when a frameshift mutation blocked translation of the transgene. Since functional β chains could not be expressed in these mice, neither from the transgene nor from the endogenous loci, they were unable to express any $\alpha\beta$TCR at all. As a consequence, maturation of thymocytes was arrested at the immature double positive ($CD4^+CD8^+$) stage and mice were completely deficient of functional $\alpha\beta$ T cells.

To further analyze the mechanism of allelic exclusion at the molecular level, transgenic mice were generated that carried a TCRβ minilocus as a substrate for gene rearrangement[69]. The TCRβ minilocus was composed of three Vβ gene segments fused to the two tandemly repeated Dβ, Jβ, Cβ clusters in germ line configuration, including the β gene enhancer. Analysis of these mice revealed that VDJ rearrangements of minilocus gene segments were specifically induced in T cells and not in B cells, indicating that the minilocus contained all sequence elements required for rearrangements. Moreover, Vβ regions encoded by the minilocus were expressed on the surface of peripheral T cells at high frequencies. Allelic exclusion can be studied in this transgenic mouse model by introducing into their germ line an already functionally rearranged TCRβ gene in addition to the minilocus. Similar experiments were reported by Ferrier et al.[18] using chimeric TCRβ minilocus-IgH constructs, in which the Cβ region and the TCRβ enhancer were replaced by the IgHCμ region including or excluding the IgH enhancer. Employing these constructs as recombination substrates in transgenic mice, they demonstrated that the IgH enhancer served as a recombination enhancer but that additional controlling elements also exist. These are apparently associated with the TCR Vβ region and provide lineage specificity of VβDβJβ assembly.

2. *Thymocyte development in TCR transgenic mice*

The combinatorial joining of different gene segments and the association of different α and β chains leads to an immature TCR repertoire that is shaped in the thymus by positive and negative selection events to render mature T cells both functional and self-tolerant. The tremendous diversity of the T cell repertoire makes it very difficult to follow a given T cell with defined specificity during ontogeny in order to study these selection processes. The transgenic mouse model, however, allows to reduce the complexity of the repertoire drastically so that a transgenic TCR of known specificity is expressed on the majority of T cells and can easily be followed throughout development with specific antibodies.

Transgenic mice were generated with functionally rearranged TCRα and β genes isolated from a cytotoxic T cell clone specific for the male antigen HY and restricted by D^b MHC class I molecules[6]. Backcrossing of the H-2^{bd} heterozygous founder mice with either strain C57L (H-2^b) or DBA/2 (H-2^d) established two transgenic lines which differed in the expression of the restriction element D^b. In addition, maturation of transgenic T cells could be studied in the presence or absence of the nominal antigen HY by comparing male and female littermates.

The results showed that T cell maturation is strictly dependent on the interaction of the TCR with thymic MHC molecules and proceeds in the absence of the nominal antigen[32, 64]. Only D^b expressing female mice supported the development of mature T cells expressing the transgenic TCR from their $CD4^+8^+$ precursors. In addition, the CD8 phenotype of the mature T cell was determined by the specificity of the TCR for class I MHC molecules.

Mature T cells bearing the transgenic receptor are found in the $CD4^-8^+$ but not in the $CD4^+8^-$ subpopulation. The results can be interpreted in terms of a regulated loss of one accessory molecule induced by the engagement of the other in the TCR/MHC interaction. However, it is also possible that loss of expression of one accessory molecule is stochastic and only appropriate TCR-coreceptor combinations are finally selected.

Positive selection of thymocytes driven by the specificity of the TCR for class I MHC molecules was also observed by Sha et al.[58]. They analyzed mice transgenic for an alloreactive $\alpha\beta$TCR specific for the L^d antigen and restricted by K^b MHC molecules. Maturation of transgenic T cells occurred only in mice expressing the K^b allele. As seen before, the bias for $CD4^-8^+$ cells in the periphery demonstrated the participation of the CD8 molecule in the selection process. With a series of H-$2K^{bm}$ mutants they found that positive selection is sensitive to minor changes in the structure of the K^b molecule, indicating that the affinity between the TCR and MHC molecules is critical for this process[37]. This concept for positive selection was confirmed using transgenic mice which carried an $\alpha\beta$TCR specific for MHC class II (I-E^k) molecules plus a peptide derived from cytochrome c[4,30]. Also in this model, transgenic thymocytes only emerged in the periphery of mice which expressed I-E^k molecules on their thymic epithelium and were found to be of the MHC class II restricted $CD4^+8^-$ phenotype. To define the requirements for positive selection more precisely, $\alpha\beta$TCR(cytochrome c/E^k) transgenic mice with defective endogenous E^k_α genes were crossed with two different E_α transgenic strains, which expressed I-E molecules specifically either in the medulla or in the cortex[35,68]. From these experiments it is clear that expression of MHC molecules on thymic cortical epithelium is essential for positive selection[4].

Tolerance induction in $\alpha\beta$TCR(HY/D^b) transgenic mice was studied in male littermates of H-2^b haplotype[31,63]. It was shown that in the presence of the nominal antigen HY and the correct restriction element D^b autoreactive thymocytes were deleted at the immature $CD4^+8^+$ stage leading to a 10-fold reduction in the total number of thymocytes. The level of CD8 expressed on thymocytes turned out to be crucial in this deletion process as T cells bearing only low amounts of CD8 together with the transgenic TCR were not eliminated and released into the periphery. In vitro experiments demonstrated that these cells were not male reactive. It was argued that the low surface density of CD8 prevented them from being autoreactive.

Immature $CD4^+8^+$ cortical thymocytes as the target for negative selection were also identified by Sha et al.[58] in their transgenic mouse model, resulting in a severe depletion of thymocytes in mice expressing the L^d antigen together with H-2^b MHC alleles. The MHC class II restricted $\alpha\beta$TCR used by Berg et al.[3] contained Vβ3 as the variable region gene of the β chain. It has been demonstrated that Vβ3 on its own conferred to the TCR an intrinsic affinity to minor lymphocyte stimulating antigens Mls-$2^a/3^a$ and caused deletion of thymocytes bearing such receptors in strains expressing these antigens[1,20,54]. This was also found for other combinations of Vβ regions and MHC class II I-E alleles and for superantigens, like staphylococcal enterotoxin[27,28,38,66,71]. In normal mice, superantigen-induced deletion takes place at the transition from the immature $CD4^+8^+$ to the mature single positive $CD4^+8^-$ or $CD4^-8^+$ stage. Transgenic mice, however, expressing a Vβ3 containing TCR together with Mls-$2^a/3^a$ antigens, showed massive deletion of autoreactive T cells already at the $CD4^+8^+$ stage in agreement with what was found in other transgenic systems. The early and high expression of TCRs in transgenic mice might be the reason for their early deletion in the thymus. Different results were obtained by Pircher et al.[52] with a transgenic TCR displaying two specificities, for lymphocytic choriomeningitis virus (LMCV) – H-$2D^b$ as well as for the Mls^a antigen. Tolerance to LMCV was shown to be induced by deletion of $CD4^+8^+$ thymocytes, whereas tolerance to Mls^a antigen was mediated by deletion of only mature thymocytes without reducing the number of immature $CD4^+8^+$ thymocytes. The maturation stage of thymocytes affected by clonal deletion seems to be antigen-dependent in this transgenic mouse model[49]. Using mice transgenic for a Vβ8.1 beta chain Blackman et al.[5] showed that a significant fraction of $CD4^+8^-$ peripheral T cells survived clonal deletion in the thymus induced by Mls-1^a antigens. As these cells were unresponsive in vitro to any stimulus tested, induction of tolerance in the thymus is not only established by clonal deletion but also by clonal anergy. Clonal anergy, rather than clonal deletion, as a tolerance mechanism induced by the Mls^a antigen was found already previously by Rammensee et al.[55] in non-transgenic mice.

Although TCR expression in transgenic mice as well as repertoire studies in normal mice clearly have identified immature cortical $CD4^+8^+$ thymocytes as targets for positive selection and induction of self-tolerance, little is known about the molecular mechanism by which the interaction of TCRs with MHC molecules in the thymus, together with the accessory molecules CD4 and CD8, either promotes further maturation or leads to cell death. It has been proposed that selection and deletion require different levels of avidity of the thymocyte/MHC interaction[36]. Alternatively, the receptor on thymocytes may couple to different signal transduction pathways between both events[41]. It is also possible that both processes are mediated by different cell types in that interaction of the thymocyte with MHC on thymic cortical epithelium induces maturation whereas interaction with MHC plus self-peptide on hemopoietic or other cells in the thymus results in deletion[44,59,70]. In addition, different versions of self-MHC plus self-peptides may be expressed on both cell types[42]. Clearly, thymic positive and negative selection need further analysis to unravel their molecular basis

and eventually identify defects that lead to failure of tolerance induction and may cause autoimmune disease.

3. *Mutations affecting thymocyte development*

Several mutations which occurred spontaneously in mice have been shown to affect thymocyte development and were used to shed more light on the processes which govern T cell maturation.

a) *scid*

Mice carrying the *scid* (severe combined immune deficiency) mutation in homozygous form are largely unable to productively rearrange their Ig and TCR loci[10] (for review see ref. 11). As a consequence, *scid* mice lack functional T and B cells and develop only a rudimentary thymus containing about 1–2% of the normal number of thymocytes. They express high levels of the Thy1 antigen, but no CD3, nor CD4 or CD8 molecules on their surface. Upon introduction of the transgenic $\alpha\beta$TCR(HY/D^b) into these mice, the thymus developed in H-2^b female littermates to about its normal size and contained about normal numbers of thymocytes, which expressed both CD4 and CD8 molecules[57]. As in non-*scid* mice, maturation of thymocytes strictly depended on the expression of the appropriate restriction element D^b in the thymus. Transgenic thymocytes of $CD4^-8^+$ phenotype matured only in D^b expressing $\alpha\beta$TCR transgenic *scid* mice, but not in D^b negative ones. Virtually no $CD4^+8^-$ cells were detectable in the thymus because the *scid* mutation prevented the generation of endogenous TCRα chains almost completely which, in association with the transgenic β chain, could be positively selected by MHC class II molecules. In the presence of the *scid* mutation, the complexity of the T cell repertoire is further reduced in $\alpha\beta$TCR transgenic mice and leads to a virtually $\alpha\beta$TCR monoclonal mouse. The results show that functionally rearranged TCR transgenes can complement the effect of the *scid* mutation on T cell development, demonstrating that it is inherent to the T cell affecting only gene rearrangement. The formation of a TCR leads to expression of CD4 and CD8 molecules and to extensive proliferation of thymocytes in *scid* mice.

A functional β transgene alone is also able to induce surface expression of CD4 and CD8 molecules on *scid* thymocytes, but is inadequate to effectively promote proliferation[70] (and H. Bluethmann, unpublished). The number of thymocytes is only slightly increased in βTCR transgenic *scid/scid* mice compared to non-transgenic littermates. They acquire a $CD4^+8^+$ phenotype but do not develop further. Surprisingly, thymocytes express the transgenic β chain on their surface, but apparently without the CD3 complex, as staining with a CD3ε-specific monoclonal antibody was negative. It seems that expansion of thymocytes is effectively triggered only by a complete $\alpha\beta$TCR/CD3 complex.

As in non-*scid* mice, thymocyte development is arrested at the $CD4^+8^+$ stage in D^b negative $\alpha\beta$TCR transgenic *scid/scid* mice. They express an $\alpha\beta$TCR/CD3 complex, however, with inappropriate specificity for positive selection on, for instance, H-2^d MHC alleles. The number of thymocytes was reduced to about 10% of the level found in D^b positive mice (H. Bluethmann, unpublished). This argues that TCR/MHC interaction not only induces maturation but also promotes proliferation of thymocytes. Although mature $CD4^-8^+$ T cells develop in the thymus of D^b positive $\alpha\beta$TCR transgenic *scid/scid* mice, their lymph nodes were clearly underdeveloped[57]. They contained about 5 times as many lymphocytes as non-transgenic *scid* mice, but still 10 times less than transgenic non-*scid* littermates. A transgenic $\alpha\beta$TCR, although promoting thymic development, is apparently not sufficient for the accumulation of normal numbers of peripheral T cells. This failure may be due to the very limited T cell repertoire in these mice.

The expression of the HY antigen and D^b molecules in male $\alpha\beta$TCR transgenic *scid/scid* mice leads, as in non-*scid* mice, to the deletion of most of their thymocytes at the $CD4^+8^+$ stage, except those with low levels of CD8. It appears that the deletion process is neither anti-idiotypic nor does it require the presence of other regulatory T cells.

b) *lpr*

The *lpr* (lymphoproliferation) mutation causes in homozygous form lymphadenopathy and autoimmunity in MRL and C57BL/6 mice[60]. The autoimmune syndrome develops with age and T cells of an unusual phenotype ($CD4^-8^-B220^+CD3^+$) accumulate in the periphery[45]. They have been shown to be of polyclonal origin[48] and may eventually constitute up to 90% of all T cells in older mice. Although the defect is inherent to T cells[29], the disease is also thymus dependent in that neonatal thymectomy markedly retards the development of the symptoms[61]. Reimplantation of a normal thymus even of different genotype restores the autoimmune syndrome[65], indicating that thymocytes carrying the *lpr* mutation can in fact develop on allogeneic thymic MHC molecules. The high frequency of autoreactive T cells in the periphery suggests a failure in thymic tolerance induction. However, T cells using Vβ segments with intrinsic affinity to Mls antigens or I-E molecules were deleted from the mature TCR repertoire also in *lpr/lpr* mice[46]. Alternatively, a defect in peripheral T cell tolerance mechanisms for the suppression of autoreactive T cell clones could be invoked as an explanation.

To gain new insight into the nature of the *lpr* mutation, the potentially autoreactive transgenic $\alpha\beta$TCR (HY/D^b) was crossed into MRL-*lpr/lpr* and C57BL/6-*lpr/lpr* mice[47]. The experiments were designed to address the question whether the overproduction of self-reactive T cells in the thymus leads to development of excessive numbers of $CD4^-8^-B220^+CD3^+$ T cells and lymphadenopathy. Unexpectedly, the presence of the transgenic TCR completely inhibited the formation of abnormal T cells and development of lymphadenopathy

in αβTCR transgenic *lpr/lpr* mice. This drastic effect was independent of the specificity of the transgenic TCR and occurred both in male and female mice, as well as in the presence or absence of D^b MHC molecules. The transgenic TCR, however, did not reduce hypergammaglobulinemia and autoantibody production, demonstrating that lymphoproliferation and autoimmunity can be dissociated in *lpr/lpr* mice. Moreover, it was shown that production of autoantibodies did not depend on the presence of $CD4^-8^-B220^+CD3^+$ T cells in *lpr/lpr* mice. T cell help for autoantibody production in B cells probably comes predominantly from the $CD4^+$ mature T cell population. Interestingly, αβTCR transgenic D^b male *lpr/lpr* mice showed an increased Ig and RF production, but not anti-DNA antibody production, compared to non-transgenic *lpr/lpr* mice. Therefore, in the transgenic *lpr/lpr* mice, some B cell help might be derived from the HY reactive and D^b restricted $CD8^+$ T cells.

The mechanism by which the transgenic TCR eliminates development of abnormal T cells and lymphadenopathy remains to be elucidated. It is possible that the early and high expression of the transgenic TCR on the majority of thymocytes overcomes an intrinsic thymic maturation defect of T cells in *lpr/lpr* mice.

Amelioration of all aspects of the autoimmune syndrome was obtained by infecting *lpr/lpr* mice with a recombinant vaccinia virus expressing the human interleukin IL-2 gene[24]. Providing the mice with a long-lasting supply of IL-2 not only reduced the titer of circulating autoantibodies but also improved thymic differentiation and suppressed the amount of abnormal $CD4^-8^-B220^+CD3^+$ T cells. Although IL-2 treatment improved all aspects of the disease, none were completely eliminated.

4. Outlook: Gene targeting in the immune system

Gene targeting by homologous recombination (for review see ref. 12) has opened up new ways to analyze the immune system. Instead of lengthy screening for useful mutations which occur spontaneously or are randomly induced, gene targeting allows the introduction of designed mutations by homologous recombination into any given gene once it is cloned. Gene targeting will be of increasing importance in dissecting the immune system further, for instance for the analysis of molecular mechanisms underlying thymic selection and peripheral tolerance.

Known genes with well-documented functions have been targeted to study the consequence of their inactivation and to generate mouse models with designed defects. Using this method, it was shown that ablation of β2-microglobulin eliminates the expression of MHC class I molecules in mice homozygous for the mutation[75]. Consequently, no mature $CD4^-8^+$ cytotoxic αβ T cells developed in these mice as no appropriate restriction elements necessary for positive selection of MHC class I restricted T cells were expressed on thymic epithelium[74]. The γδ T cell lineage, however, seems to be unaffected by the mutation. Interestingly, embryonic development proceeds normally, showing that MHC class I molecules are not essential for embryogenesis[16]. In addition, β2-microglobulin is not a necessary chemotactic molecule[50] for the attraction of T cell precursors to the thymus as normal numbers of thymocytes develop in mutant mice. More recently, additional genes have been addressed. Interleukins, for example, are known to play a key role in T cell activation. Deletion mutants will be of invaluable help in further clarifying their function in vivo.

Most importantly, homologous recombination will give cloning of hitherto unknown genes new impetus. They may be identified by differential screening for developmental or stage specific genes. Once they have been cloned, designed mutations may be introduced by homologous recombination to analyze the processes they are involved in.

Gene targeting by homologous recombination does not necessarily imply ablation of the targeted gene but it allows the introduction of more subtle modifications up to the exchange of a few nucleotides.

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1 Abe, R., Vacchio, M. S., Fox, B., and Hodes, R., Preferential expression of the T cell receptor Vβ3 gene by Mls^c reactive T cells. Nature *335* (1988) 827–830.
2 Alt, F., Enea, V., Bothwell, A. L., and Baltimore, D., Activity of multiple light chain genes in murine myeloma cells producing a single, functional light chain. Cell *21* (1980) 1–12.
3 Berg, L. J., Fazekas de St Groth, B., Pullen, A. M., and Davis, M. M., Phenotypic differences between αβ versus β T cell receptor transgenic mice undergoing negative selection. Nature *340* (1989) 559–562.
4 Berg, L. J., Pullen, A. M., Fazekas de St Groth, B., Mathis, D., Benoist, C., and Davis, M. M., Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. Cell *58* (1989) 1035–1046.
5 Blackman, M. A., Gerhard-Burgert, H., Woodland, D. L., Palmer, E., Kappler, J. W., and Marrack, P., A role for clonal inactivation in T cell tolerance to $Mls\text{-}1^a$. Nature *345* (1990) 540–542.
6 Bluethmann, H., Kisielow, P., Uematsu, Y., Malissen, M., Krimpenfort, P., Berns, A., von Boehmer, H., and Steinmetz, M., T-cell-specific deletion of T-cell receptor transgenes allows functional rearrangement of endogenous α and β genes. Nature *334* (1988) 156–159.
7 Bonneville, M., Janeway, C. A. J., Ito, K., Haser, W., Ishida, I., Nakanishi, N., and Tonegawa, S., Intestinal intraepithelial lymphocytes are a distinct set of γδ T cells. Nature *336* (1988) 479–481.
8 Born, W., Happ, M. P., Dallas, A., Reardon, C., Kubo, R., Shinnick, T., Brennan, P., and O'Brien, R., Recognition of heat shock proteins and γδ cell function. Immunol. Today *11* (1990) 40–43.
9 Born, W., Harris, E., and Hannum, C., Ontogeny of T-cell receptor gene expression. Trends Genet. *3* (1987) 132–136.
10 Bosma, G. C., Custer, M. J., and Bosma, M. J., A severe combined immunodeficient mutation in the mouse. Nature *301* (1983) 527–530.
11 Bosma, M. J., Phillips, R. A., and Schuler, W., The scid mouse. Characterization and potential uses, in: Current Topics in Microbiology and Immunoloy, vol. 152. Springer Verlag, Berlin 1989.
12 Capecchi, M. R., The new mouse genetics: altering the genome by gene targeting. Trends Genet. *5* (1989) 70–76.
13 Chou, H. S., Behlke, M. A., Godambe, S. A., Russell, J. H., Brooks, C. G., and Loh, D. Y., T cell receptor genes in an alloreactive CTL clone: implications for rearrangement and germline diversity of variable gene segments. EMBO J. *5* (1986) 2149–2155.
14 Chou, H. S., Nelson, C. A., Godambe, S. A., Chaplin, D. D., and Loh, D. Y., Germline organization of the murine T cell receptor β-chain genes. Science *238* (1987) 545–548.

15 Coleclough, C., Nature *303* (1983) 23.
16 Dargemont, C., Dunon, D., Deugnier, M.-A., Denoynelle, M., Girault, J.-M., Thymotaxin, a chemotactic protein, is identical to β2-microglobulin. Science *246* (1989) 803–806.
17 Davis, M. M., and Bjorkman, P. J., T-cell antigen receptor genes and T-cell recognition. Nature *334* (1988) 395–402.
18 Ferrier, P., Krippl, B., Blackwell, T. K., Furley, A. J., Suh, H., Winoto, A., Cook, W. D., Hood, L., Costantini, F., and Alt, F. W., Separate elements control DJ and VDJ rearrangement in a transgenic recombination substrate. EMBO J. *9* (1990) 117–125.
19 Fondell, J. D., Marolleau, J. P., Primi, D., and Marcu, K. B., On the mechanism of non-allelically excluded Vα-Jα T cell receptor secondary rearrangements in a murine T cell lymphoma. J. Immun. *144* (1990) 1094–1103.
20 Fry, A. M., and Matis, L. A., Self-tolerance alters T-cell receptor expression in an antigen-specific MHC restricted immune response. Nature *335* (1988) 830–832.
21 Furutani, M., Yanagi, Y., Fujisawa, I., Nakayama, T., Kishimoto, H., Kuida, K., Asano, Y., and Tada, T., Post-transcriptional allelic exclusion of two functionally rearranged T cell receptor alpha genes. Int. Immun. *3* (1989) 281–288.
22 Goodman, T., and Lefrancois, L., Expression of the γδ T-cell receptor on intestinal $CD8^+$ intraepithelial lymphocytes. Nature *333* (1988) 855–858.
23 Goverman, J., Minard, K., Shastri, N., Hunkapiller, T., Hansburg, D., Sercarz, E., and Hood, L. E., Rearranged β T-cell receptor genes in a helper T-cell clone specific for lysozyme. Cell *40* (1985) 859–867.
24 Gutierrez-Ramos, J. C., Andreu, J. L., Revilla, Y., Vinuela, E., and Martinez, C., Recovery from autoimmunity of MRL/*lpr* mice after infection with an interleukin-2/*vaccinia recombinant* virus. Nature *346* (1990) 271–274.
25 Havran, W. L., and Allison, J. P., Developmentally ordered appearance of thymocytes expressing different T cell antigen receptors. Nature *335* (1988) 443.
26 Janeway, C. A., Jones, B., and Hayday, A., Specificity and function of T cells bearing γδ receptors. Immunol. Today *9* (1988) 73.
27 Kappler, J. W., Staerz, U., White, J., and Marrack, P., Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature *322* (1988) 35–40.
28 Kappler, J. W., Wade, T., White, J., Kushnir, E., Blackman, M., Bill, J., Roehm, N., and Marrack, P., A T-cell receptor Vβ segment that imparts reactivity to a class II Major Histocompatibility Complex product. Cell *49* (1987) 263–271.
29 Katagiri, T., Cohen, P. L., and Eisenberg, R. A., The *lpr* gene causes an intrinsic T cell abnormality that is required for hyperproliferation. J. exp. Med. *167* (1988) 741.
30 Kaye, J., Hsu, M. L., Sauron, M. E., Jameson, S. C., Gascoigne, N. R. J., and Hedrick, S. M., Selective development of $CD4^+$ T cells in transgenic mice expressing a class II MHC-restricted antigen receptor. Nature *341* (1989) 746–749.
31 Kisielow, P., Bluethmann, H., Staerz, U. D., Steinmetz, M., and von Boehmer, H., Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes. Nature *333* (1988) 742–746.
32 Kisielow, P., Teh, H. S., Bluethmann, H., and von Boehmer, H., Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. Nature *335* (1988) 730–733.
33 Krimpenfort, P., Ossendorp, F., Borst, J., Melief, C., and Berns, A., T cell depletion in transgenic mice carrying a mutant gene for TCRβ. Nature *341* (1989) 742–746.
34 Kuziel, W. A., Takashima, A., Bonhadi, M., Bergstresser, P. R., Allison, J. P., Tigelaar, R. E., and Tucker, P. W., Regulation of T-cell receptor γ-chain RNA expression in murine $Thy\text{-}1^+$ dendritic epidermal cells. Nature *328 (1987)* 263–266.
35 LeMeur, M., Gerlinger, P., Benoist, C., and Mathis, D., Correcting an immune-response deficiency by creating Eα gene transgenic mice. Nature *316* (1985) 38–42.
36 Lo, D., Ron, Y., and Sprent, J., Induction of MHC-restricted specificity and tolerance in the thymus. Immun. Res. *5* (1986) 221.
37 Loh, D. Y., Sha, W. C., Nelson, C. A., Newberry, R. D., Kranz, D. M., and Russell, J. H., Positive and negative selection of T lymphocytes. Cold Spring Harb. Symp. quant. Biol. *54* (1989) 147–151.
38 MacDonald, H. R., Schneider, R., Lees, R. K., Howe, R. C., Acha-Orbea, H., Festenstein, H., Zinkernagel, R. M., and Hengartner, H., T cell receptor Vβ use predicts reactivity and tolerance to Mls^a-encoded antigens. Nature *332* (1988) 40–45.
39 Malissen, M., Trucy, J., Letourneur, F., Rebai, N., Dunn, D. E., Fitch, F. W., Hood, L., and Malissen, B., A T cell clone expresses two T cell receptor α genes but uses one αβ heterodimer for allorecognition and self MHC-restricted antigen recognition. Cell *55* (1988) 49.
40 Marolleau, J. P., Fondell, J. D., Malissen, M., Trucy, J., Barbier, E., Marcu, K. B., Cazenave, P. A., and Primi, D., The joining of germline Vα to Jα genes replaces the preexisting Vα-Jα complexes in a T cell receptor αβ positive T cell line. Cell *55* (1988) 291–300.
41 Marrack, P., Blackman, M., Burgert, H. G., McCormack, J. M., Cambier, J., Finkel, T. H., and Kappler, J., T-cell repertoire and thymus. Cold Spring Harbor Symp. quant. Biol. *54* (1989) 105–110.
42 Marrack, P., and Kappler, J., The T-cell receptor. Science *238* (1987) 1073–1079.
43 Matis, L. A., Ezquerra, A., and Coligan, J. E., Expression of two distinct T cell receptor α/β heterodimers by an antigen-specific T cell clone. J. exp. Med. *168* (1988) 2379–2384.
44 Matzinger, P., and Guerder, S., Does T cell tolerance require a dedicated antigen presenting cell? Nature *338* (1989) 74–76.
45 Morse, H. D., Davidson, W. F., Yetter, R. A., Murphy, E. D., Roths, J. B., and Coffmann, R. L., Abnormalities induced by the mutant gene *lpr*: expansion of a unique lymphocyte subset. J. Immun. *129* (1982) 2612–2618.
46 Mountz, J. D., Smith, T. M., and Toth, K. S., Altered expression of self-reactive T cell receptor Vβ regions in autoimmune mice. J. Immun. *144* (1990) 2159–2166.
47 Mountz, J. D., Zhou, T., Eldridge, J., Berry, K., and Bluethmann, H., Transgenic rearranged T-cell receptor gene inhibits lymphadenopathy and accumulation of $CD4^-CD8^-B220^+$ T cell in *lpr*/*lpr* mice. J. exp. Med. *172* (1990) 1805–1817.
48 Nemazee, D. A., Studer, S., Steinmetz, M., Dembic, Z., and Kiefer, M., The lymphoproliferating cells of MRL-*lpr*/*lpr* mice are a polyclonal population that bear the T lymphocyte receptor for antigen. Eur. J. Immun. *15* (1985) 760.
49 Ohashi, P. S., Pircher, H., Buerki, K., Zinkernagel, R. M., and Hengarter, H., Distinct sequence of negative or positive selection implied by thymocyte T-cell receptor densities. Nature *346* (1990) 861–863.
50 Ohno, S., The original function of MHC antigens as the general plasma membrane anchorage site of organogenesis-directed proteins. Immun. Rev. *33* (1977) 59.
51 Perry, R. P., Kelley, D. E., Coleclough, C., Seidman, J. G., Leder, P., Tonegawa, S., Matthyssens, G., and Weigert, M., Transcription of mouse κ chain genes: implications for allelic exclusion. Proc. natl Acad. Sci. USA *77* (1980) 1937–1941.
52 Pircher, H., Buerki, K., Lang, R., Hengartner, H., and Zinkernagel, R. M., Tolerance induction in double specific T-cell receptor transgenic mice varies with antigen. Nature *342* (1989) 559–561.
53 Pircher, H., Ohashi, P., Miescher, G., Lang, R., Zikopoulos, A., Buerki, K., Mak, T. W., MacDonald, H. R., and Hengartner, H., T cell receptor (TcR) beta chain transgenic mice: studies on allelic exclusion and on the Tcr + gamma/delta population. Eur. J. Immun. *20* (1990) 417–424.
54 Pullen, A. M., Marrack, P., and Kappler, J. W., The T cell repertoire is heavily influenced by tolerance to polymorphic self antigens. Nature *335* (1988) 796–801.
55 Rammensee, H.-G., Kroschewski, R., and Frangoulis, B., Clonal anergy induced in mature $V\beta6^+$ lymphocytes on immunizing $Mls\text{-}1^b$ mice with $Mls\text{-}1^a$ expressing cells. Nature *339* (1989) 541–544.
56 Schittek, B., Unkelbach, E., and Rajewsky, K., Violation of allelic exclusion of the T cell receptor β genes in a helper T cell clone. Int. Immun. *1* (1989) 273–280.
57 Scott, B., Bluethmann, H., Teh, H. S., and von Boehmer, H., The generation of mature T cells requires interaction of the αβ T-cell receptor with major histocompatibility antigens. Nature *338* (1989) 591–593.
58 Sha, W. C., Nelson, C. A., Newberry, R. D., Kranz, D. M., Russell, J. H., and Loh, D. Y., Positive and negative selection of an antigen receptor on T cells in transgenic mice. Nature *336* (1988) 73–76.
59 Shimonkevitz, R. P., and Bevan, M. J., Split tolerance induced by intrathymic adoptive transfer of thymocyte stem cells. J. exp. Med. *158* (1988) 143.
60 Steinberg, A. D., Ravech, E. S., Laskin, C. A., Smith, H. R., Santoro, T. J., Miller, M. L., and Plotz, P., Systemic lupus erythematosus: insights from animal models. Ann. int. Med. *100* (1984) 714–734.
61 Steinberg, A. D., Roths, J. D., Murphy, E. D., Steinberg, R. T., and Raveche, E. S., Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/p-*lpr*/*lpr* mice. J. Immun. *124* (1980) 871–873.

62 Stingl, G., Gunter, K. C., Tschachler, E., Yamada, H., Lechler, R. I., Yokoyama, W. M., Steiner, G., Germain, R. N., and Shevach, E. M., Thy-1$^+$ dentritic epidermal cells belong to the T cell lineage. Proc. natl Acad. Sci. USA *84* (1987) 2430–2434.

63 Teh, H. S., Kishi, H., Scott, B., and von Boehmer, H., Deletion of autospecific T cells in T cell receptor (TCR) transgenic mice spares cells with normal TCR levels and low levels of CD8 molecules. J. exp. Med. *169* (1989) 795–806.

64 Teh, H. S., Kisielow, P., Scott, B., Kishi, H., Uematsu, Y., Bluethmann, H., and von Boehmer, H., Thymic major histocompatibility complex antigens and the $\alpha\beta$ T cell receptor determine the CD4/CD8 phenotype of T cells. Nature *335* (1988) 229–233.

65 Theofilopoulos, A. N., Balderas, R. S., Shawler, D. L., Lee, S., and Dixon, F. J., The influence of thymic genotype on the systemic lupus erythematosus like disease and T cell proliferation of MRL/Mp-*lpr*/*lpr* mice. J. exp. Med. *153* (1981) 1405–1414.

66 Tomonari, K., and Lovering, E., T-cell receptor-specific monoclonal antibodies against a Vβ11-positive mouse T-cell clone. Immunogenetics *28* (1988) 445.

67 Uematsu, Y., Ryser, S., Dembic, Z., Borgulya, P., Krimpenfort, P., Berns, A., von Boehmer, H., and Steinmetz, M., In transgenic mice the introduced functional T cell receptor β gene prevents expression of endogenous β genes. Cell *52* (1988) 831–841.

68 van Ewijk, W., Ron, Y., Monaco, J., Kappler, J., Marrack, P., LeMeur, M., Gerlinger, P., Durand, B., Benoist, C., and Mathis, D., Compartmentalization of MHC class II gene expression in transgenic mice. Cell *53* (1988) 357–370.

69 van Meerwijk, J. P. M., Bluethmann, H., and Steinmetz, M., T-cell specific rearrangement of T-cell receptor β transgenes in mice. EMBO J. *9* (1990) 1057–1062.

70 von Boehmer, H., Kishi, H., Borgulya, P., Scott, B., van Ewijk, W., Teh, H. S., and Kisielow, P., Control of T-cell development by the TCR$\alpha\beta$ for antigen. Cold Spring Harbor Symp. quant. Biol. *54* (1989) 111–118.

71 White, J., Herman, A., Pullen, A. M., Kubo, R., Kappler, J. W., and Marrack, P., The Vβ specific superantigen Staphylococcal enterotoxin B: Stimulation of mature T cells and clonal deletion in neonatal mice. Cell *56* (1989) 27–35.

72 Winoto, A., Mjolsness, S., and Hood, L., Genomic organization of the genes encoding mouse T-cell receptor α-chain. Nature *316* (1985) 832–836.

73 Yancopoulos, G. D., and Alt, F. W., Regulation of the assembly and expression of variable-region genes. A. Rev. Immun. *4* (1986) 339–368.

74 Zijlstra, M., Bix, M., Simister, N. E., Loring, J. M., Raulet, D. H., and Jaenisch, R., β2-microglobulin deficient mice lack CD4$^-$8$^+$ cytolytic T cells. Nature *344* (1990) 742–746.

75 Zijlstra, M., Li, E., Sajjadi, F., Subramani, S., and Jaenisch, R., Germ-line transmission of a disrupted β2-microglobulin gene produced by homologous recombination in embryonic stem cells. Nature *342* (1989) 435–438.

0014-4754/91/090884-07$1.50 + 0.20/0