Analysis of the immune system with transgenic mice: B cell development and lymphokines

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Abstract. Over the last decade transgenic mice expressing genes relevant for the immune system have been generated. Transgenic expression of immunoglobulin heavy and/or light chain genes of different isotypes and different specificities have helped to better understand phenomena relevant to B cell development such as allelic exclusion of immunoglobulins and B cell tolerance. Transgenic mice expressing interleukin genes have also been used to study the ways of action of these important growth and differentiation factors in the context of the mouse immune system.
Key words. Transgenic mice; immune system; Ig allelic exclusion; B cell tolerance; lymphokines.

Control of immunoglobulin (Ig) gene rearrangement in Ig transgenic mice

B lymphocytes produce and secrete antibodies and are thus responsible for the humoral immune response. The genes encoding immunoglobulins (Ig) are composed of several exons corresponding to the constant (C) region and two exons encoding the variable portion (V) of the antibody. The latter ones are not fixed genetic entities as the C exons but are built somatically from separated gene segments that are joined by a recombination process during maturation of B lymphocytes. The joining process, known as Ig gene rearrangement, occurs in an ordered and regulated fashion: first gene segments encoding the variable regions of the Ig heavy (H) chain, namely the V (variable), D (diversity) and J (joining) elements, are appended to generate a V_HDJ_H complex, before the corresponding gene elements of the light (L) chain gene, namely V_L and J_L segments, are rearranged (see ref. 59 for a review). Every B cell, being diploid, contains a set of genes encoding Ig's on each allele and yet only one of the two Ig alleles is ever functionally rearranged and therefore expressed. This phenomenon is known as 'allelic exclusion'[44]. The low probability for two functional rearrangements to occur in the same B cell was claimed to explain allelic exclusion[7]. However, the frequent finding of incomplete, DJ_H, rearrangements on the second allele of B cell hybridomas suggested an active feedback where the signalling is possibly exerted by the product of the first functional rearrangement[2]. Transgenic mice provide a valid system to address this question and so, functionally rearranged Ig genes were among the first to be expressed in transgenic mice. In mice carrying functionally rearranged transgenic H chain genes, expression of endogenous H chain genes is impaired[20,40,48,62]. Suppression of endogenous H chain gene expression apparently results from inhibition of gene rearrangement at the IgH loci because in transgenic pre-B and B cells, very few complete V_HDJ_H rearrangements were found. Most H chain alleles are found to be either unrearranged or only partially rearranged[20,48,54,62]. Allelic exclusion of endogenous H chain genes is observed in mice expressing transgenic H chains of isotypes as different as murine[48,54,62] and human μ[40], murine δ[20], γ_{2b}[55] and α[27]. Furthermore, transgenic mice expressing only the membrane form of μ (μ_m) show allelic exclusion of endogenous H chains, whereas mice expressing only the secretory form of μ (μ_s) do not[5,28,41]. These findings suggest an active, μ_m-induced mechanism rather than unspecific interference of H chains in the process of Ig gene rearrangement. Little is known about the biochemical basis of this signalling event but at least two pre-B cell specific proteins (called ω and τ) have been shown to associate with μ[45] and are proposed to form surrogate light chains[60] and be involved in signalling in pre-B cells[29].

The time point of the transcriptional onset of transgenic H chain genes is not known and may differ between different transgenic lines, due to different chromosomal integration sites. However, it is conceivable that transgenic H chain gene expression can occur at pre-B cell stages as early as the ones characterized by transcription of endogenous unrearranged V_H genes[64] and hence before endogenous H chain genes had rearranged. Thus, in transgenic pre-B cells, H chains are probably produced earlier than in normal pre-B cells. Since μ_m has been shown to play a key role in the induction of L gene rearrangement[46] in pre-B cells, early production of transgenic H chains probably leads to early (endogenous) L chain gene rearrangement and production, resulting in complete Ig molecules. Therefore, in H chain gene transgenic mice, feedback inhibition of endogenous H chain gene rearrangement could result from early expression of either the transgenic H chain alone or the complete Ig protein. The analysis of L chain gene transgenic mice has helped to elucidate this question.

When functionally rearranged L chain κ genes are expressed in transgenic mice, allelic exclusion of endogenous κ chains is observed[47]. Coexpression of transgenic and endogenous κ chains can be found in B cell hybridomas from κ transgenic mice but only in those cases lacking H chain expression. Expression of transgenic κ and endogenous H chain was always accompanied by the lack of rearrangements at the endogenous κ locus[47]. These results suggested that L chains alone are not able to inhibit rearrangement and that complete Ig molecules are necessary to signal cessation of rearrangement at the L chain locus.

B lymphocytes express either κ (about 95% of spleen B cells) or λ (5% of splenic B cells) light chains but never both at the same time. The mechanisms governing 'isotypic exclusion' are poorly understood. Extensive rearrangements leading to deletion of the Cκ exon are shown to accompany and probably precede λ gene rearrangement and expression [32, 33, 51]. In transgenic mice expressing functionally rearranged λ transgenes, expression of endogenous κ genes is suppressed to a great extent, although the number of B cells expressing transgenic λ diminish with age and give way to κ-expressing B lymphocytes [16, 38]. As seen for allelic exclusion, isotypic exclusion in λ transgenic mice results from inhibition of rearrangement at the endogenous κ locus, although in both cases a variable fraction of B cells is able to escape this inhibition and produce endogenous Ig's [5, 16, 20, 38, 48, 53]. Moreover, both in κ- and λ-transgenic mice unrearranged H chain genes are found in B cell hybridomas [16, 38]. These observations have led to the conclusion that unrearranged and functionally rearranged H chain genes can normally coexist in pre-B cells [16]. The production of endogenous (or transgenic) H chains would inhibit H chain gene rearrangement at the V_H to DJ_H but not at the D to J_H step [16, 46], possibly by termination of transcription at the unrearranged V_H loci [64], and would induce rearrangement at the κ gene locus [46]. Therefore, D to J_H rearrangement could continue during L chain gene rearrangement and only upon productive L chain gene rearrangement, resulting in a complete Ig (H + L) molecule, would recombinase activity be shut off. Early expression of transgenic H chain genes would turn off transcription of unrearranged V_H germline elements and induce rearrangement at the κ locus. If at all, only DJ_H rearrangements could occur and only during the time needed to achieve a functional L chain gene rearrangement. Then, rearrangement at all Ig gene loci would be prevented by complete Ig. If instead, a transgenic L chain gene is expressed early in pre-B cells, rearrangement of endogenous H chain genes would generally not be inhibited and only a successful rearrangement of the first H chain allele would lead to a shut-off of recombinase activity through association with transgenic L chains before any rearrangement could happen at the second H chain allele. Additionally, inhibition of rearrangement at the endogenous L gene locus would occur in most L chain transgenic B cells. Such a model could explain most of the findings made on the rearrangement status of endogenous Ig genes in Ig transgenic mice but still fails to explain the recurrent observation of B cells escaping allelic exclusion in IG transgenic mice.

B cell development in Ig transgenic mice

Mice homozygous for the *scid* (severe combined immune deficiency) mutation are characterized by a defect in the ability to generate functional V(D)J rearrangements at the Ig and T cell receptor (TCR) loci [3]. Therefore, development of B and T lymphocytes is arrested at an early stage of differentiation in these mice. Expression of a functional μ transgene in *scid* mice leads to a rescue of pre-B cells (defined as those bone marrow cells positive for the B lineage specific surface antigen B220 and negative for surface IgM, sIgM), whereas transgenic (μ + κ) expressing *scid* mice show rescue of bone marrow pre-B ($B220^+$, $sIgM^-$) as well as of mature B cells ($B220^+$, $sIgM^+$) [4]. The results indicate that expression of μ or IgM is needed for the developmental transition from pro-B to pre-B and from pre-B to mature B cells, respectively. However, expression of transgenic μ + κ on the surface of bone marrow mature B cells is not sufficient to fully reconstitute the peripheral B cell compartment in *scid* mice [4]. A similar observation is made with *scid* mice expressing transgenic TCRαβ genes (see H. Blüthmann, this issue). In both cases this finding might relate to the complexity of lymphocyte development and could indicate that more than just one (transgenic) Ig- or TCR-specificity is needed.

The importance of an intact μ H chain for B cell development has been studied in transgenic mice expressing truncated μ genes. A human heavy chain disease (HCD)-like μ transgene lacking the entire VDJ exon has been shown to be able to mediate allelic exclusion of endogenous murine H chain genes (Corcos, D., Iglesias, A., Buchini, D., and Jami, J., submitted for publication). However, the truncated HCD-like μ protein is apparently not able to drive complete B cell differentiation, since transgenic spleen B lymphocytes cannot be stimulated with polyclonal B cell activators like bacterial lipopolysaccharide (LPS) to proliferate and secrete Ig's. In contrast to intact μ H chains, a mutant murine H chain μ lacking the exon encoding for the first constant domain (μΔ1) is not able to induce L chain gene rearrangement in pre-B cells [21]. Transgenic mice expressing the μΔ1 gene construct show a strong depletion of B lymphocytes. In addition, both splenic B lymphocytes and B cell hybridomas from these mice co-express low levels of the transgene together with normal levels of endogenous H chains. Furthermore, and in contrast to wild-type μ transgenic mice, neonatal μΔ1 transgenic mice are completely devoid of B cells (A. Iglesias, unpublished data). These data suggest impaired development of B cells expressing the transgenic truncated μΔ1 protein, possibly due to its incapacity to efficiently trigger L chain gene rearrangement.

Obstruction of B cell development is also observed in 'normal' Ig transgenic mice. In one case, mice transgenic for μ_m or (μ_m + κ) genes with specificity for the hapten 4-hydroxy-3-nitrophenyl (NP) or for an anti-NP idiotype, respectively, showed a strong depletion of pre-B and B cells, with no detectable levels of transgenic Ig expression [34]. In another case, transgenic expression of a μ gene with specificity for NP resulted in a strong depletion of bone marrow pre-B cells and of splenic B cells of the 'conventional', bone marrow-derived, B cell lineage,

whereas the peritoneum-derived (Ly1) B cell lineage was unaffected[18]. In contrast, transgenic mice expressing ($\mu + \kappa$) transgenes with specificity for the hapten 2,4,6-trinitrophenyl (TNP) have only slightly reduced numbers of pre-B and B cells both in bone marrow and spleen[14]. Similar results are obtained with transgenic mice expressing the anti-TNP μ gene only (our unpublished observations). Whereas differences in the extent of B lymphocyte depletion found in different lines of transgenic mice expressing the same transgene could be due to positional effects and/or different levels of transgenic expression[53] the different potential of different transgenic Ig's in generating a conventional B cell compartment might be related to restrictions imposed by their particular specificity. In fact, in splenic B cell hybridomas derived from transgenic mice expressing the anti-TNP μ gene alone, the transgenic μ chain is preferentially found associated with κ chains very similar to the κ chain originally expressed in the anti-TNP hybridoma Sp6, from which the transgenic μ gene is derived. Up to 70% of the transgenic hybridomas are TNP specific and 50–60% express endogenous V_κ genes that are highly homologous with, or belong to the same V_κ gene family as the Sp6-κ gene (V_κ19), as if regeneration of the original or a very similar V_H/V_L combination were advantageous for transgenic B cell development (A. Iglesias, manuscript in preparation). Similar findings were made in mice expressing μ or κ transgenes derived from an anti-phosphorylcholin (PC)-specific hybridoma. In the spleen of these mice the corresponding κ or μ genes originally expressed in the anti-PC specific hybridoma, which were not injected in μ or κ transgenic mice, respectively, are highly expressed in the absence of PC-immunization[54]. Furthermore, in two cases analyzed, the B cells that evade allelic exclusion and express endogenous Ig's in μ or ($\mu + \kappa$) transgenic mice represent a small proportion of bone marrow B cells. These cells become a larger fraction of resting, surface IgM^+ B cells in the spleen and constitute the majority of Ig-secreting splenic B cells[10, 14]. Therefore, the few B cells spared from allelic exclusion are preferentially stimulated and expanded, thus providing the immune system with a 'natural' repertoire of specificities not accounted for by the transgene[14]. As is the case for the natural repertoire in normal mice, endogenous Ig-expressing B cells in Ig-transgenic mice are probably also selected and expanded on the basis of cross-reactive auto-specificities[14]. Perhaps the differences in the capacity to generate a B cell compartment observed among different Ig transgenic mice are actually reflecting the intrinsic abilities of the different transgenic specificities to elicit and form part of a connective system of cross-reactive idiotypic specificities. In *scid* ($\mu + \kappa$) transgenic mice formation of such a cross-reactive idiotypic network would be impossible because endogenous Ig genes cannot rearrange functionally and thus the peripheral B cell compartment cannot be reconstituted. Some observation on μ transgenic mice are indeed indicative of such network interactions between transgenic and endogenous antibodies.

In two independent mouse lines transgenic for the μ gene derived from an anti-NP specific hybridoma[62], the idiotype of the original hybridoma was present at higher levels than normal in transgenic serum in the absence of any immunization. Moreover, B cell hybridomas from unimmunized transgenic mice carried this idiotype at unexpectedly high frequencies (>60%), as compared with the low frequencies (<1%) found in hybridomas of unimmunized normal mice. Since a high proportion of the transgenic hybridomas did not express the transgene, and since the idiotype and even the transgenic V region was found associated with the endogenous μ allotype or even with non-μ isotypes, endogenous antibodies must have accounted for the high incidence of transgenic idiotype and NP-specificity found[63]. Apparently, in these transgenic mice, expression of the transgene has influenced the specificity and idiotype of a large fraction of the endogenous antibodies in a way that remains yet to be determined. However, alternative explanations are possible and have indeed been provided for similar findings made in other transgenic mice. In transgenic mice expressing a μ transgene with specificity for the hapten p-azaphenylarsonate (Ars), a large proportion of the Ars-specific antibodies made by Ars-immunized animals are composed of the transgenic V_H region associated to IgG rather than to IgM[9]. Sequence analysis of Ig mRNA from Ars-specific transgenic hybridomas showed that the transgenic V_HDJ_H gene segment is directly linked to the γ constant region sequences of isotypes γ1 and γ2a. The findings suggested that a switch recombination event has occurred in these mice between transgenic and endogenous Ig genes[9]. Since similar results were obtained in four different transgenic lines integration of the transgene within the endogenous μ locus on chromosome 12 is very unlikely. Indeed, interchromosomal switch recombination has been demonstrated in hybridomas of these mice that joined the μ switch sequences of the transgene with the switch sequences of the endogenous Cγ's. Translocation of chromosome 12 to chromosome 5, where the transgene has integrated, was the consequence[11]. Therefore, interchromosomal 'trans-switch' recombination in the B cells of Ars-μ transgenic mice account for the majority of the Ars-specific IgG antibodies found in these animals upon Ars immunization. Likewise, in transgenic mice expressing a human μ_m gene, the transgenic V_HDJ_H gene segment is found linked to an endogenous murine constant γ1 sequence[50]. This hybrid mRNA sequence has been proposed to result from a hitherto unknown 'trans-splicing' event that occurs in the absence of immunization and results in simultaneous expression of human μ and murine IgG proteins on the surface of some transgenic B cells[50].

Finally, somatic mutations have been detected within the V region of a PC-specific κ transgene upon immunization with PC[42]. This result clearly demonstrated that the pro-

cess of hypermutation of Ig genes is not dependent on rearrangement nor is it restricted to a specific chromosomal localization[42]. Somatic mutation of Ig genes is essential for the generation of antibodies with high affinity for antigen during the secondary immune response[59]. Ig-transgenic mice could therefore prove useful in the study of the mechanisms governing the process of somatic hypermutation of Ig genes.

In summary, transgenic mice expressing functionally rearranged Ig genes have not only served to better understand allelic exclusion but may also prove an adequate system to study B cell maturation and the formation and internal dynamics of antibody repertoire.

Transgenic models for B cell tolerance

Transgenic mice expressing Ig's with specificities directed against mouse self components have also been helpful in the study of the mechanisms governing tolerance of B lymphocytes.

In transgenic mice expressing anti-MHC class I ($\mu + \kappa$) antibodies, B lymphocytes are clonally deleted in the lymphoid organs[36]. Clonal deletion takes place only in transgenic mice that express the H-2 haplotype recognized by the transgenic antibodies (K^k) and occurs early in the bone marrow, probably at the transition from pre-B to mature B cells[37].

Clonal deletion of autoreactive B cells has also been observed in transgenic mice expressing a μ gene with specificity for the T cell specific surface antigen CD8[5] (Brombacher, F., personal communication). Additionally, in this case ectopic expression of the transgene in thymus leads to a severe depletion of immature thymocytes, as CD8 is expressed by the majority of immature thymocytes[5].

Besides clonal deletion, non-deletional mechanisms have also been demonstrated to efficiently induce peripheral tolerance. Using double transgenic mice that express both transgenic hen egg lysozyme (HEL) and a pair of H and L anti-HEL Ig genes, clonal anergy of autoreactive, peripheral B lymphocytes has been demonstrated[12]. Since the transgenic H gene included the VDJ-Cμ as well as the downstream Cδ regions, both transgenic isotypes, IgM and IgD, were expressed in transgenic mice, due to alternative splicing. Anti-HEL single transgenic mice secrete high amounts of the transgenic antibodies but production of anti-HEL antibodies is suppressed in double transgenic mice expressing both transgenic anti-HEL and sufficiently high levels of transgenic HEL. B lymphocytes expressing the transgenic Ig's on their surface are not deleted nor reduced in the double transgenic mice. Instead, a persisting state of anergy is induced in the B lymphocytes of (HEL; anti-HEL) double transgenic mice, that is characterized by down regulation of transgenic surface IgM but not surface IgD and the intrinsic inability of most double transgenic B cells to secrete antibodies[1, 13]. Thus, for both types of tolerance induction, clonal deletion and non-deletional anergy induction, transgenic mice provide valid experimental models which facilitate the study of the mechanisms involved in unresponsiveness of B lymphocytes against self components.

Immunological consequences of transgenic lymphokine expression

Interleukins (IL) are essential components of the immune system as they promote growth and differentiation of leukocytes and are crucial in the formation of immune and inflammatory responses[31]. The intricate ways of their action, often exhibiting pleiotropic properties as well as synergistic cooperation, make the study of their functions in in vitro systems rather incomplete and often contradictory. Transgenic mice (over-) expressing interleukin genes represent valid tools for the study of interleukin function in the context of the entire immune system.

Granulocyte-macrophage-colony stimulating factor (GM-CSF) is a T cell derived cytokine that stimulates the proliferation of macrophages, granulocytes and eosinophils[30]. Transgenic mice overexpressing the murine GM-CSF gene show increased concentrations of serum GM-CSF and augmented number of macrophages in lymphoid tissues. Macrophage accumulation in the lens and retinal tissue, as well as in the pleural and peritoneal cavities and in striated muscles is observed in these mice. Activation of the accumulated macrophages results in pathological damage of the retinal tissue and blindness, as well as in muscle wasting and premature death[25].

IL-2 is mainly produced by T lymphocytes and is a potent mitogenic factor for T cells, but also for B cells, natural killer cells and lymphokine-activated killer cells[31]. Deregulated expression of human IL-2 in transgenic mice has also been reported[22, 23]. Expression of the human IL-2 gene under the control of an H-2 promoter in transgenic mice leads to alopecia, pneumonia and an increase in the number of $Thy1^+$ dendritic epidermal cells, while immune responses of spleen cells were impaired[22]. In the other case, the expression of human IL-2 driven by a mouse metallothionein-I promoter, led to motor ataxia due to lymphocyte infiltration into and destruction of the cerebellum[23]. In both cases, the mechanisms leading to the described phenotypes are unknown.

Transgenic mice have also been generated that express the small subunit of the human IL-2 receptor (Tac, IL-2Rα)[15, 39]. The transgene is expressed mainly in lymphoid tissues and in one case, early expression of the human IL-2Rα gene resulted in increased numbers of $Thy1^-$ thymocyte precursors and depletion of ($CD4^+CD8^+$) double positive thymocytes[15].

IL-4 is a growth factor for B and T lymphocytes and plays a central role in the induction of Ig isotype switch leading to IgE synthesis in murine B lymphocytes[52].

Overexpression of IL-4 under the transcriptional control of μ H chain gene promoter and enhancer in transgenic mice resulted in premature death. Viable IL-4 transgenic mouse lines could only be established using DNA constructs in which transcription of the IL-4 gene is attenuated[57]. In these mice depletion of double positive thymocytes was observed, thus supporting the postulated role of IL-4 in T cell development[43]. Additionally, increased levels of serum IgE and inflammatory ocular lesions characteristic of allergic reactions have been observed in these mice. The occular lesions were the consequence of lymphocyte and macrophage infiltration and, interestingly, in some mice they develop also in the absence of increased levels of serum IgE[57]. The IL-4 transgenic mice may thus prove an invaluable model to study allergy.

Other transgenic mice have been developed that express the murine IL-4 gene under the control of the *Ick* gene promoter, such that transgenic IL-4 expression is confined to the thymus[26]. These mice also show disturbed T cell development, in particular a strong depletion of double positive thymocytes and peripheral T cells. However, in this case, no B cell anomalies or increased levels of serum IgE or IgG1 were observed[26].

Finally, low levels of transgenic IL-4 expression have also been reported[35]. In this case, though, only hyperexpression of MHC class II genes in B cells, but no constitutive B cell activation, nor any gross lymphoid or immune perturbation was observed.

Taken together the data obtained with IL-4 transgenic mice demonstrate the importance of local expression of IL-4 in different microenvironments both for T lymphocyte differentiation and effector functions.

Overexpression of IL-5 in T lymphocytes of IL-5 transgenic mice has recently been reported[8,58]. The IL-5 transgenic mice are characterized by continuous blood and spleen eosinophilia, increased levels of serum IL-5 and eosinophil infiltration of transgenic lung and gut tissues. Nonetheless, the mice remain normal and show no sign of tissue damage. Normally, IL-5 is produced by T cells upon parasite infestation. IL-5 has been shown to promote eosinophil generation in bone marrow cultures. In these systems though, other lymphokines like IL-1, IL-3 and GM-CSF have also been implicated in eosinophil production[6,61]. However, in the bone marrow of IL-5 transgenic mice eosinophil generation is enhanced and no increased levels of IL-3 or GM-CSF are found[8]. That the observed effects directly result from transgenic IL-5 overexpression was demonstrated in experiments where administration of anti-IL-5 or anti-IL-5 receptor antibodies to the transgenic mice was sufficient to abolish eosinophilia[19]. These findings indicate that IL-5 is sufficient for induction of eosinophilia and that additional factors, possibly antigen-antibody formation, are required for degranulation and tissue damaging.

IL-6 is a growth factor for plasmacytoma and myeloma cells, where its production is deregulated[24]. It also induces terminal differentiation of B lymphocytes and expression of acute phase proteins in hepatocytes[24]. When human IL-6 was expressed in transgenic mice[56], the high levels of serum IL-6 provoked an extensive plasmacytosis in lymphoid organs, with infiltration of plasma cells in lung, liver and kidney. This phenotype is accompanied by a 120- to 400-fold increased level of serum IgG1. However, the extensive plasmacytosis did not lead to plasmacytoma formation and the plasma cells were shown not to contain a translocation of the *c-myc* oncogene often found in plasmacytomas[24]. The results suggest that IL-6 is a potent stimulator of cell division and maturation of B cells but cannot be the sole factor that induces malignant transformation of plasma cell, the latter probably being the result of a second event such as a rare *c-myc* translocation.

IL-7 is a growth factor derived from bone marrow stromal cells that stimulates proliferation of immature B- and T-lymphocytes and of mature T cells if a second stimulus is provided[17]. Transgenic expression of the mouse IL-7 gene under the control of the H gene enhancer and L gene promoter has been shown to induce preferential proliferation of B cell precursors[49]. The distribution of all other lymphocyte subsets is not affected, but their absolute numbers are increased in most transgenic mice. In contrast, the absolute number of granulocytes and macrophages is not increased, thus confirming the specificity of the growth action of IL-7 on precursor lymphocytes.

Taken together, transgenic expression of cytokines provide in vivo models where the multiple in vitro effects assigned to these potent factors can be tested in a more systemic way. In most cases (GM-CSF, IL-4, IL-5, IL-6 and IL-7) overexpression of interleukins in transgenic animals resulted in a more restricted pattern of stimulated target cells than it had been previously observed in vitro. In no case the preferential proliferation of a defined leukocyte subset has led to tumor formation, thus making these transgenic mice appropriate tools for studying interleukin action in vivo. Additionally, crossing of the available transgenic mice expressing individual IL-genes may also help to elucidate the cooperative ways of action often observed for interleukins. The generation via gene targeting in embryonic stem (ES) cells of mice carrying disrupted interleukin genes will further help to clarify their specific roles within the interwoven network of cytokine communication.

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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-