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Review

Extrathymic Pathways of T Cell Differentiation

TORU ABO*

Department of Immunology, Niigata University School of Medicine, Niigata, Japan

Abstract. It is known that the liver is a major hematopoietic organ at fetal stages, but the hematopoiesis of this organ ceases at birth. However, the liver is still found to comprise c-kit$^+$ stem cells and gives rise to extrathymic T cells, NK cells, and even granulocytes after birth. Extrathymic T cells generated in the liver of mice are identified as intermediate TCR (TCR^{int}) cells, which include the NK1.1$^+$TCR^{int} (i.e. NKT cells) and NK1.1$^-$TCR^{int} subsets. Although extrathymic T cells are few in number during youth, they increase in number with advancing age. The number and function of extrathymic T cells are also elevated under conditions of stress, infections, malignancy, pregnancy, autoimmune diseases, chronic GVH diseases, etc. Under these conditions, the mainstream of T cell differentiation in the thymus, which produces conventional T cells, is inversely suppressed. Extrathymic T cells comprise self-reactive forbidden clones and mediate cytotoxicity against abnormal self-cells. Therefore, they might be beneficial for the elimination of such cells. However, over-activation of extrathymic T cells might be responsible for the onset of certain autoimmune diseases.

Key words: extrathymic T cells; intermediate TCR cells; NKT cells; liver; autoimmune diseases.

Introduction

We have been characterizing the nature of extrathymic T cells in the liver of humans and mice[34, 58, 60, 67, 78, 82]. We initiated these studies since we found that unusual T cell populations, such as double-negative (DN) CD4$^-$8$^-$ αβT cells or CD8$^+$ γδT cells, are abundant in the liver of mice[2, 55, 56, 69]. These T cell populations also exist in the liver of congenitally athymic nude mice[31, 68]. We speculated that these T cells might be generated through extrathymic pathways and that the liver might be one of the major sites for the extrathymic pathways of T cell differentiation. We have determined that both DN αβT cells and CD8$^+$ γδT cells in the liver share common properties, including the phenotypes of IL-2Rβ$^+$, intermediate TCR (TCR^{int}), NK1.1$^+$ (approximately 50%), CD44$^+$, L-selectin$^-$, etc.[4, 57, 70]. We term them TCR^{int} cells. Based on these criteria, conventional T cells (i.e. thymus-derived T cells) are termed TCR^{high} cells, carrying the phenotypes of IL-2Rβ$^-$, TCR^{high}, NK1.1$^-$, CD44$^-$, L-selectin$^+$, etc., under resting conditions.

In parallel with these studies, two series of murine studies on unusual T cells were ongoing. First, many investigators observed and characterized DN CD4$^-$8$^-$ αβT cells with or without the expression of NK1.1 antigen. A small number of these cells were found to be present in the thymus[11, 15, 18, 20, 21, 41, 63, 64, 77, 87, 90] and periphery, e.g. the spleen[36, 45, 65, 66, 72], lymph nodes[24, 30] and bone marrow[62]. Subsequently, BENDELAC

Abbreviations used: TCR^{int} cells – intermediate TCR cells, TCR^{high} cells – high TCR cells, NKT cells – natural killer T cells, DN – double-negative, DP – double-positive.

* Correspondence to: Dr. Toru Abo, Department of Immunology, Niigata University School of Medicine, Asahimachi-dori 1, Niigata 951-8510, Japan, fax: +81 25 227 0766, e-mail: immunol2@med.niigata-u.ac.jp

et al.[7, 9] reported that some of these T cells express the NK1.1 antigen and that these NK1.1$^+$ αβT cells, including DN and CD4$^+$ αβT cells, recognize non-MHC gene-encoded MHC class I-like molecules, CD1d. These T cells are termed natural killer T (NKT) cells. In another series of studies by TANIGUCHI and his colleagues, it was found that some suppressor T cell hybridomas preferentially use an invariant TCRα chain, Vα14-Jα281, and that such Vα14$^+$ T cells are detected in the liver, thymus and other organs[39, 75]. They also demonstrated that most Vα14$^+$ T cells, if not all, coincide with NKT cells and are extrathymically generated in the liver[43].

NKT cells belong to a subset of TCR^{int} cells, i.e. TCR^{int} cells consist of both NK1.1$^+$ and NK1.1$^-$ subsets at a ratio of 1:1, especially in the liver and thymus[80, 82]. Moreover, it has been found that TCR^{int} cells (including NKT cells) are generated extrathymically in the liver and through an alternative intrathymic pathway[18, 73]. In contrast, conventional T cells are generated through the mainstream of T cell differentiation in the thymus. In light of these findings, we further characterize TCR^{int} cells and NKT cells in the liver and other organs in terms of their phenotype, morphology, function and development in this review.

Tissue Distribution of TCR^{int} Cells and NKT Cells

Two-color staining for CD3 and IL-2Rβ or for CD3 and NK1.1 was conducted to identify TCR^{int} (CD3^{int}) cells or NKT cells in mice. NK cells were identified as IL-2Rβ$^+$CD3$^-$, TCR^{int} cells as IL-2Rβ$^+$CD3^{int}, and conventional T cells as IL-2Rβ$^-$CD3^{high}. Both NK cells and TCR^{int} cells consistently express IL-2Rβ, while conventional T cells lack the expression of IL-2Rβ[82]. It is conceivable that conventional T cells acquired a resting form of lymphocytes in phylogeny (i.e. IL-2Rα$^-$β$^-$). After antigenic stimulation they begin to express a high affinity IL-2R (i.e. IL-2Rα$^+$β$^+$). Reflecting this situation, primitive lymphocytes respond more quickly than others (NK cells→TCR^{int} cells→TCR^{high} cells)[59]. Among these, however, the magnitude of response is the greatest in TCR^{high} cells.

The distribution of CD3^{int} cells and NKT cells in various organs was almost the same, namely, both were abundant in the liver but few in number in other organs. However, the proportion of NKT cells was always smaller than that of TCR^{int} cells in all tested organs. Concerning the fact that NKT cells had a lower density of TCR-CD3 complex (i.e. TCR^{int} cells), NKT cells are present within the population of TCR^{int} cells. The pro-

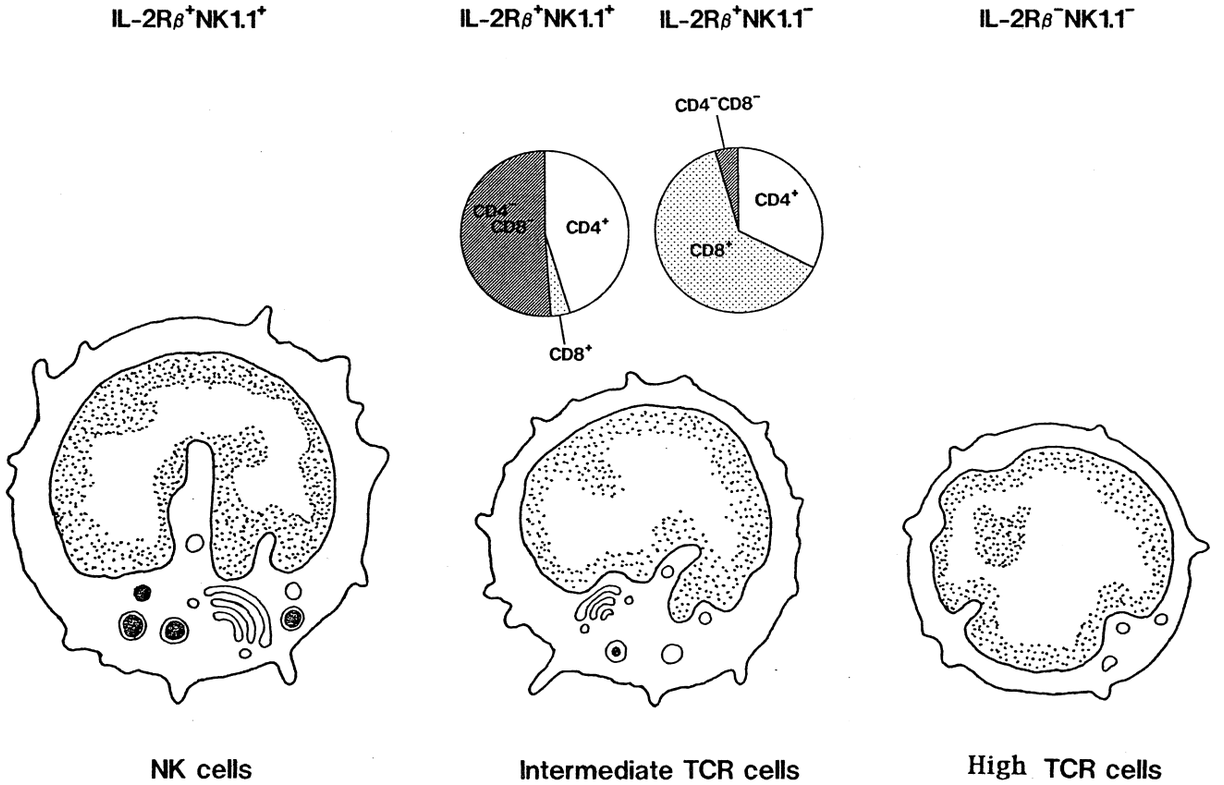

Fig. 1. Phylogenetic development of lymphocytes of a NK/T cell lineage. TCR^{int} cells comprise IL-2Rβ$^+$NK1.1$^+$ and IL-2Rβ$^+$NK1.1$^-$ subsets

portion of NKT cells among TCR^{int} cells was >50% in the liver and thymus, whereas that of NKT cells among TCR^{int} cells was approximately 20% in the spleen, lymph nodes and bone marrow[82].

Characterization of the Phenotype of NK Cells, TCR^{int} Cells and TCR^{high} Cells

A possible line of the phylogenic development of lymphocytes of a NK/T cell lineage is represented: NK cells→TCR^{int} cells→TCR^{high} cells (Fig. 1). Depending on the development, lymphocytes acquired a resting form of morphology and phenotype. According to this change, well-developed T cells have the morphology of small resting lymphocytes and acquire a long life-span. Once they encounter corresponding antigens, they undergo cell-division to expand the clones. It is presumed that this kind of clonal expansion is not accompanied by NK cells and is only slightly accompanied by TCR^{int} cells.

The subsets of TCR^{int} cells comprise cells with a distinct phenotype (see the top of Fig. 1). The IL-2Rβ^+NK1.1$^+$ subset (i.e. NKT cells) mainly consists of DN $CD4^-8^-$ cells and $CD4^+$ cells, whereas the IL-2Rβ^+NK1.1$^-$ subset mainly consists of $CD8^+$ cells[34]. It is speculated that the IL-2Rβ^+NK1.1$^+$ subset is a more primitive form than the IL-2Rβ^+NK1.1$^-$ subset. Interestingly, it has been reported that the former subset, namely NKT cells, recognize CD1 molecules (i.e. a non-classical or monomorphic MHC class I-like antigen)[7, 9]. On the other hand, the $CD8^+$IL-2Rβ^+NK1.1$^-$ cells among TCR^{int} cells seem to recognize some peptides together with a polymorphic MHC class I antigen. In the case of athymic nude mice, TCR^{int} cells and NKT cells are few in number, although all of them are classified as TCR^{int} cells[31, 68]. Moreover, the majority of both NKT cells and NK1.1$^-$$TCR^{int}$ cells express the $CD8^+$ phenotype instead of the DN or $CD4^+$ phenotype.

Differentiation of TCR^{int} Cells or NKT Cells in the Liver and Thymus

It is interesting to know how TCR^{int} cells or NKT cells are generated in the bodies of mice. Since all T cells in congenitally athymic nude mice are TCR^{int} cells, it is obvious that TCR^{int} cells are generated extrathymically[31, 68]. In both athymic mice and normal eu-

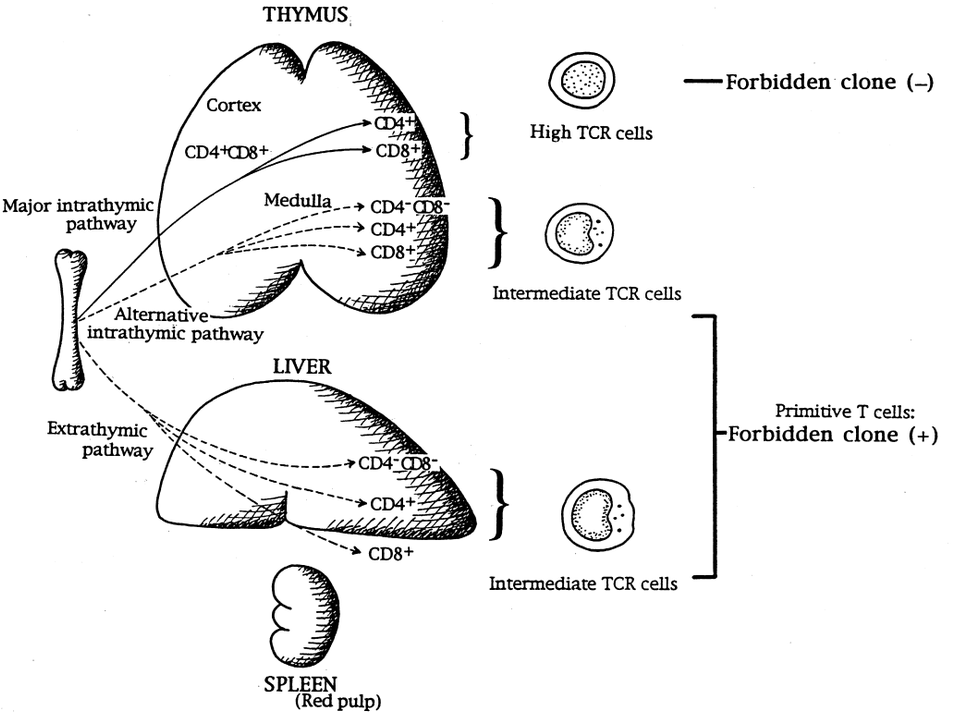

Fig. 2. Pathways of T cell differentiation in the thymus and liver. The major intrathymic pathway produces conventional T cells through a stage of DP $CD4^+8^+$ cells. This pathway eliminates forbidden clones by negative selection. On the other hand, an alternative intrathymic pathway and the extrathymic pathways in the liver (and spleen) produce primitive T cells which contain forbidden clones due to incomplete negative selection

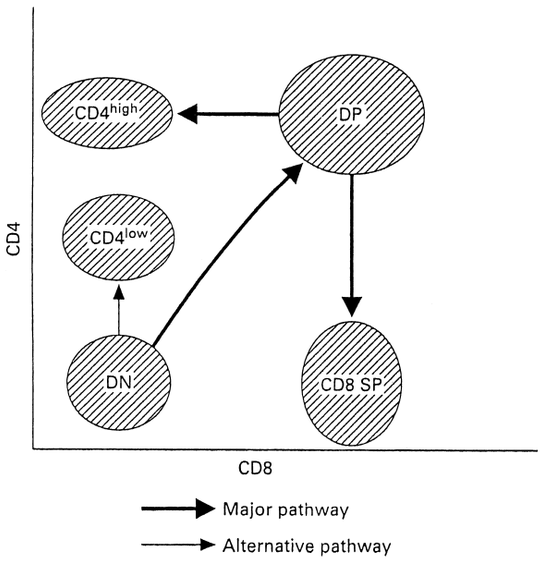

Fig. 3. Schema for the major pathway (mainstream) and alternative pathway of T cell differentiation in the thymus. The thymus comprises both the major pathway (the mainstream) and an alternative pathway of T cell differentiation: the alternative pathway becomes rather prominent when mice are recovering from acute thymic atrophy

thymic mice, the major site appears to be the liver (Fig. 2). We have demonstrated that c-kit$^+$ stem cells exist in the parenchymal space of the liver and that such stem cells give rise to extrathymic T cells, forming clusters of many lymphoid cells[54, 83]. These c-kit$^+$ cells have the potential to give rise not only to lymphoid cells, but also to other hematopoietic cells such as granulocytes and erythrocytes[83, 88]. Extrathymic T cells and granulocytes, but not erythrocytes, are generated in the parenchymal space of the liver and then migrate to the sinusoidal lumen after maturation[78, 89]. IL-7 functions as a cytokine for the initial maturation of extrathymic T cells[48], but IL-12, IL-15 and IL-18 may function as cytokines for the functional maturation[6, 23, 26].

TCR^{int} cells or NKT cells are also generated through an alternative intrathymic pathway (Fig. 2 top). Although a few TCR^{int} cells and NKT cells are scattered throughout the cortex region, the majority of these T cells exists in the medullary region[38]. On the other hand, the mainstream of T cell differentiation for the generation of conventional T cells occurs in the cortex region. Similar to the case of extrathymic T cells in the liver, TCR^{int} cells and NKT cells in the thymus are generated into three subsets, i.e. DN CD4$^-$8$^-$, CD4$^+$ and CD8$^+$, without passing through a double-positive (DP) CD4$^+$8$^+$ stage[44]. Since TCR^{high} cells and NKT cells are generated without passing through a DP CD4$^+$8$^+$ stage, their negative selection for the elimination of forbidden clones is always incomplete, as determined in the Mls system[49, 51, 60]. To detect the forbidden clones in the Mls system, we have to use mice other than C57BL/6 (B6) mice or (B6×other strains) F_1 mice.

The alternative pathway of T cell differentiation in the thymus is minor in youth. However, this pathway becomes prominent when the hosts recover from acute thymic atrophy (Fig. 3). DN CD4$^-$8$^-$ TCR$^-$ cells become CD4^{low}TCR^{int} cells or DN TCR^{int} cells[44]. More importantly, these TCR^{int} cells generated through the alternative pathway contain self-reactivity. In these experiments, we induced acute thymic atrophy by irradiation (6.5 Gy) or by the administration of hydrocortisone (10 mg/mouse). In addition of these conditions, it is speculated that some microbial infections or physical (or mental) stress could induce acute thymic atrophy. We propose the possibility that the generation of self-reactive TCR^{int} cells might be intimately associated with the onset of autoimmune diseases. The prominence of TCR^{int} cells during recovery from thymic atrophy is due to the recovery of the TCR^{int} cells, which is quicker than that of conventional TCR^{high} cells. It should be emphasized that there was no leakage of forbidden clones into conventional T cells at this time[44, 49, 51]. Irrespective of NK1.1$^+$TCR^{int} cells or NK1.1$^-$TCR^{int} cells, the association of these primordial T cells should be concerned with the mechanism underlying autoimmune diseases.

Independent Generation of Extrathymic T Cells in Various Sites

TCR^{int} cells or NKT cells are also generated extrathymically in the spleen (possibly the red pulp region) (Fig. 2). The extrathymic pathways for the generation of TCR^{int} cells and NKT cells occur at multiple sites in the body[34]. Such sites include the uterus[27, 28, 40, 42], small intestine[16, 17, 19, 25, 52], and exocrine glands such as the salivary glands[74] and lacrimal glands. In the small intestine, CD8$\alpha\beta$ $\gamma\delta$T cells and DP CD4$^+$8$^+$ $\alpha\beta$T cells are uniquely present. All of these extrathymic T cells lack the expression of NK1.1 antigen. In certain phylogenic periods, extrathymic T cells in the liver and small intestine develop in a similar way, but then develop independently. In the case of the appendix, an unusual population of T cells (i.e. B220$^+$T cells) seen in autoimmune MRL-*lpr*/*lpr* mice are present[88]. This T cell population might also be extrathymic T cells.

A final question in this section is how c-kit$^+$ stem cells are supplied to the liver. To answer this question,

we prepared B6.Ly5.1 and B6.Ly5.2 strains of parabiotic mice[71, 74, 76]. By gated analysis in immunofluorescence tests, we found that TCR^{int} cells and NKT cells in the liver showed a low mixture of partner cells after parabiosis. On the other hand, conventional T cells in all peripheral immune organs (e.g. the spleen and lymph nodes) became a half-and-half mixture of a mouse's own cells and his partner's cells by 14 days after parabiosis. This is due to the fact that conventional T cells in the spleen and lymph nodes come from another site, the thymus. If c-kit^+ stem cells in the liver are consistently supplied from the bone marrow, TCR^{int} cells and NKT cells should have mixed well with partner cells. However, this was not the case. It is concluded that c-kit^+ stem cells in the liver are not supplied from the bone marrow under normal conditions after birth. Of course, if c-kit^+ stem cells are impaired in the liver (e.g. by irradiation), such stem cells are efficiently replaced by those of the bone marrow[71].

A similar low mixture of partner cells was seen even in the thymus[76]. In other words, c-kit^+ stem cells in the thymus are not consistently supplied from the bone marrow after birth. It is speculated that such stem cells existing in the thymus might home to the thymus at fetal stages as well.

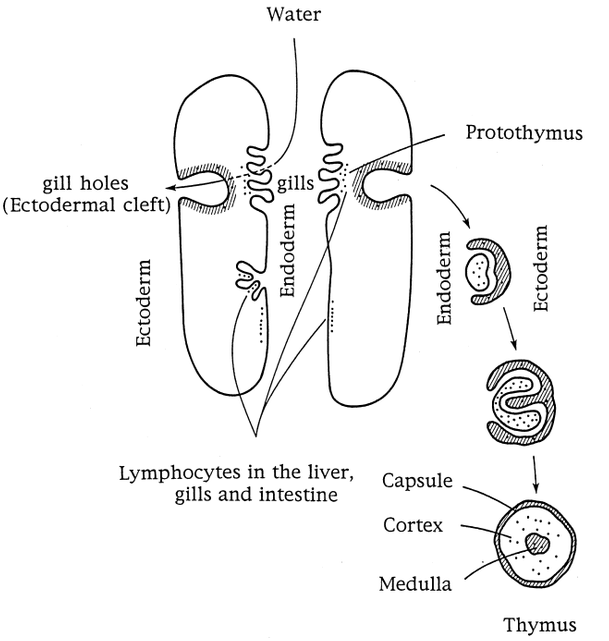

Fig. 4. Diagram of how the immune organs developed in phylogeny. The primary immune system might have developed in the digestive tract and the ectoderm. Subsequently, the immune system was distributed to the liver, which originated from the intestine. The thymus originated from a combination of the gills with the ectodermal cleft

Phylogeny of the Immune System

The concept of extrathymic T cells has given us a clearer picture of how the immune system was developed (Fig. 4). The fundamental host defense system in underdeveloped living creatures might have consisted only of macrophages. However, some groups of macrophages developed into lymphoid cells which lost their phagocytic function but acquired an immune system by use of some adhesion molecules (e.g. the immunoglobulin gene superfamily). Such sites for the development of the immune system might be the digestive tract (or intestine) and the ectoderm. Indeed, the upper portion in the digestive tract gave rise to the prototothymus (i.e. the gills and lymphoid cells under the gills), whereas the lower portion in the digestive tract gave rise to the intestinal immune system. Since the liver developed from the intestine, both the liver and intestine became the sites for the presence of primitive lymphocytes such as NK cells and extrathymic T cells. In the case of extrathymic T cells in the small intestine, almost all T cells at this site lost a NK marker, NK1.1: NKT cells are absent in the small intestine of mice. Morover, they acquired a high density of TCR (TCR^{high}) and, if not completely, acquired the DP $CD4^+8^+$ phenotype in the case of αβT cells[16, 17, 19, 25, 52]. This unique development of T cells which occurred in the intestine might be due to the high frequency of antigenic stimulation at this site. On the other hand, extrathymic T cells in the liver and even in the large intestine still comprise T cells with the NK marker (i.e. NKT cells)[57]: these organs still carry a less-developed subset of extrathymic T cells.

In the case of the protothymus, the gill holes (i.e. the ectodermal cleft) combined with the gills when living creatures went onto land. Therefore, the intact thymus always consists of ectodermal tissues[3]. Such sites are the capsule and medulla of the thymus. These ectodermal tissues are extremely important for negative selection to eliminate self-reactive clones. This schema (Fig. 4) also explains the reason why the medullary region comprises an alternative intrathymic pathway for T cell differentiation: the medulla, which is derived from the ectoderm, still contains the ancient extrathymic pathway of ectodermal (skin) origin. Even in mice and humans, many extrathymic T cells are present under the skin. If there is a genetic abnormality of the skin, the formation of the thymus itself is also impaired, as shown in congenitally athymic nude mice.

Antigen Recognition by NK Cells and NKT Cells

It is known that NK cells mainly recognize MHC antigen-losing self-cells (e.g. malignant tumor cells) while NKT cells recognize some self-antigens in the context with monomorphic MHC class I (-like) antigens. These monomorphic MHC class I (-like) antigens include a non-MHC-encoded MHC class I-like molecule, CD1d, and a MHC-encoded MHC class I antigen, T lymphocytes[8, 33, 84]. On the other hand, human monomorphic MHC class I (-like) antigens include CD1 antigens (including CD1a, CD1b, CD1c, and CD1d) and non-classical HLA class I antigens (including HLA-E, -F, and –G). There is another subset, $NK1.1^{-}$ TCR^{int}, among TCR^{int} cells[82]. This subset seems to recognize some self-antigens in the context of both monomorphic and polymorphic MHC class I antigens. Details of the function of these $NK1.1^{-}TCR^{int}$ cells remain to be further investigated.

In the case of humans, $CD56^{+}$T cells may correspond to $NK1.1^{+}TCR^{int}$ cells, while $CD57^{+}$T cells may correspond to $NK1.1^{-}TCR^{int}$ cells[53, 60, 78]. This notion is based on their cytotoxic function and cell distribution, e.g. $CD56^{+}$T cells have potent cytotoxicity, and in the fact that $CD56^{+}$T cells are mainly generated in the liver, but $CD57^{+}$T cells are mainly generated in the bone marrow. When patients with leukemia receive bone marrow transplantation after chemotherapy, newly recovered T cells are known to be $CD57^{+}$T cells[22, 29, 32]. All of these T cells with NK markers in mice and humans have the morphology of granular lymphocytes[47, 82].

When $NK1.1^{+}TCR^{int}$ cells (NKT cells) recognize some self-antigens, almost all of the antigen presenting cells seem to use the CD1d molecule[7, 9]. In this case, one such antigen is a glycolipid, α-galactosylceramide (α-GalCer)[10, 12]. The CD1d molecule presents not only antigens such as α-GalCer, but also some peptide antigens to NKT cells[79]. It is reported that the CD1d molecule interacts with NK cell inhibitory receptors (NKIR or Ly-49A) and presents suppressive signals of the cytotoxic function to NK cells[13]. In addition to a cross relationship between NKT cells and the CD1d molecule, almost all of these NKT cells use an invariant chain of Vα14Jα281 for TCRαβ[39, 43, 75]. However, some NK^{-} T cells using non-Vα14Jα281 chains have been found to recognize some self-antigens in the context of the CD1d molecule[14].

Reciprocal Change of the Differentiation in TCR^{int} Cells and TCR^{high} Cells

The number of TCR^{int} cells and NKT cells is extremely low in the liver and other organs at birth[31]. These lymphocytes become detectable in the liver and thymus approximately 4 weeks after birth in mice. Thereafter, the number and proportion of TCR^{int} cells and NKT cells increase with advancing age, especially in parallel with thymic involution[80]. In other words, the extrathymic pathway of T cell differentiation in the liver and an alternative intrathymic pathway of T cell differentiation appear to be inversely regulated in comparison with the mainstream of T cell differentiation in the thymus. More importantly, this is not only the case in aging, but also in the cases of infections[1, 2, 59], stress[50, 81], pregnancy[37, 46], malignancy[35], chronic GVH diseases[61, 85, 86], etc. Even in youth, these conditions induce acute thymic atrophy and simultaneously augment the differentiation of TCR^{int} cells in the liver and thymus. It is conceivable that this switching of the immune system might be important in overcoming the above-mentioned emergencies in hosts, in that the autoreactive cytotoxicity mediated by TCR^{int} cells and NKT cells might be essential for the elimination of abnormal self-cells which are generated under the conditions of emergency. However, if these responses are continued for too long or are too strong, the generation of TCR^{int} cells or NKT cells might be over-activated. Since these primordial T cells always comprise self-reactive forbidden clones and mediate self-reactive cytotoxicity[34, 51, 82], such hosts are in danger of falling victim to autoimmune diseases[5, 85]. We have to emphasize that, even under these immunosuppressive conditions, there is no leakage of forbidden clones into conventional T cells (i.e. TCR^{high} cells) in the thymus and periphery[44, 49, 51]. In other words, the autoreactive responses might be events mediated only by TCR^{int} cells, including their subsets of $NK1.1^{+}TCR^{int}$ cells and $NK1.1^{-}TCR^{int}$ cells.

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References

1. ABO T., KUSUMI A., SEKI S., OHTEKI T., SUGIURA K., MASUDA T., RIKIISHI H., IIAI T. and KUMAGAI K. (1992): Activation of extrathymic T cells in the liver and reciprocal inactivation of intrathymic T cells by bacterial stimulation. Cell. Immunol., **142**, 125–136.
2. ABO T., OHTEKI T., SEKI S., KOYAMADA N., YOSHIKAI Y.,

MASUDA T., RIKIISHI H. and KUMAGAI K. (1991): The appearance of T cells bearing self-reactive T cell receptor in the livers of mice injected with bacteria. J. Exp. Med., **174**, 417–424.
3. ABO T., WATANABE H., SATO K., IIAI T., MORODA T., TAKEDA K. and SEKI S. (1995): Extrathymic T cells stand at an intermediate phylogenetic position between natural killer cells and thymus-derived T cells. Nat. Immun., **14**, 173–187.
4. ARAI K., IIAI T., NAKAYAMA M., HASEGAWA K., SATO K., OHTSUKA K., WATANABE H., HANYU T., TAKAHASHI H. E. and ABO T. (1995): Adhesion molecules on intermediate TCR cells. I. Unique expression of adhesion molecules, $CD44^+$L-selectin$^-$, on intermediate TCR cells in the liver and the modulation of their adhesion by hyaluronic acid. Immunology, **84**, 64–71.
5. ARAI K., YAMAMURA S., HANYU T., TAKAHASHI H. E., UMEZU H., WATANABE H. and ABO T. (1996): Extrathymic differentiation of resident T cells in the joints of mice with collagen-induced arthritis. J. Immunol., **157**, 5170–5177.
6. BARBULESCU K., BECKER C., SCHLAAK J. F., SCHMITT E., MEYER ZUM BUSCHENFELD K.-H. and NEURATH M. F. (1998): IL-12 and IL-18 differentially regulate the transcriptional activity of the human IFN-γ promoter in primary $CD4^+$ T lymphocytes. J. Immunol., **160**, 3642–3648.
7. BENDELAC A. (1995): Positive selection of mouse NK^+ T cells by CD1-expressing cortical thymocytes. J. Exp. Med., **182**, 2091–2096.
8. BENDELAC A. (1995): CD1: presenting unusual antigens to unusual T lymphocytes. Science, **269**, 185–186.
9. BENDELAC A., LANTZ O., QUIMBY M. E., YEWDELL J. W., BENNINK J. R. and BRUTKIEWICZ R. R. (1995): CD1 recognition by mouse NK^+ T lymphocytes. Science, **268**, 863–865.
10. BROSSAY L., NAIDENKO O., BURDIN N., MATSUDA J., SAKAI T. and KRONENBERG M. (1998): Structural requirements for galactosylceramide recognition by CD1-restricted NK T cells. J. Immunol., **161**, 5124–5128.
11. BUDD R. C., MIESCHER G. C., HOW R. C., LEES R. K., BRON C. and MACDONALD H. R. (1987): Developmentally regulated expression of T cell receptor β chain variable domains in immature thymocytes. J. Exp. Med., **166**, 577–582.
12. BURDIN N., BROSSAY L., KOEZUKA Y., SMILEY S. T., GRUSBY M. J., GUI M., TANIGUCHI M., HAYAKAWA K. and KRONENBERG M. (1998): Selective ability of mouse CD1 to present glycolipids: α-galactosylceramide specifically stimulates Vα 14^+NK T lymphocytes. J. Immunol., **161**, 3271–3281.
13. CHANG C. S., BROSSAY L., KRONENBERG M. and KANE K. P. (1999): The murine nonclassical class I major histocompatibility complex-like CD1.1 molecule protects target cells from lymphokine-activated killer cell cytolysis. J. Exp. Med., **189**, 483–491.
14. CHIU Y.-H., JAYAWARDENA J., WEISS A., LEE D., PARK S.-H., VARSAT A. D. and BENDELAC A. (1999): Distinct subsets of CD1d-restricted T cells recognize self-antigens loaded in different cellular compartments. J. Exp. Med., **189**, 103–110.
15. CRISPE I. N., MOORE M. W., HUSMANN L. A., SMITH L., BEVAN M. J. and SHIMONKEVITZ R. P. (1987): Differentiation potential of subsets of $CD4^-8^-$ thymocytes. Nature, **329**, 336–339.
16. DE GEUS B., VAN DEN ENDEN M., COOLEN C., NAGELKERKEN L., VAN DEN HIJDEN P. and ROZING J. (1990): Phenotype of intraepithelial lymphocytes in euthymic and athymic mice: implications for differentiation of cells bearing a CD3-associated γδ T cell receptor. Eur. J. Immunol., **20**, 291–298.
17. EBERT E. C. (1990): Intraepithelial lymphocytes: interferon-gamma production and suppressor/cytotoxic activities. Clin. Exp. Immunol., **82**, 81–85.
18. EGERTON M. and SCOLLAY R. (1990): Intrathymic selection of murine TCR $\alpha\beta^+$ $CD4^-CD8^-$ thymocytes. Int. Immunol., **2**, 157–163.
19. FERGUSON A. and PARROTT D. M. V. (1972): The effect of antigen deprivation on thymus-dependent and thymus-independent lymphocytes in the small intestine of the mouse. Clin. Exp. Immunol., **12**, 477–488.
20. FOWLKES B. J., KRUISBEEK A. M., TON-THAT H., WESTON M. A., COLIGAN J. E., SCHWARTZ R. H. and PARDOLL D. M. (1987): A novel population of T-cell receptor αβ-bearing thymocytes which predominantly expresses a single Vβ gene family. Nature, **329**, 251–254.
21. GOFF L. K. and HUBY R. D. J. (1992): Characterization of constitutive and strain-dependent subsets of $CD45RA^+$ cells in the thymus. Int. Immunol., **4**, 1303–1311.
22. GOROCHOV G., DEBRE P., LEBLOND V., SADAT-SOWTI B., SIGAUX F. and AUTRAN B. (1994): Oligoclonal expansion of $CD8^+CD57^+$ T cells with restricted T-cell receptor β chain variability after bone marrow transplantation. Blood, **83**, 587–595.
23. GRABSTEIN K. H., EISENMAN J., SHANEBECK K., RAUCH C., SRINIVASAN S., FUNG V., BEERS C., RICHARDSON J., SCHOENBORN M. A., AHDIEH M., JOHNSON L., ALDERSON M. R., WATSON J. D., ANDERSON D. M. and GIRI J. G. (1994): Cloning of a T cell growth factor that interacts with the β chain of the interleukin-2 receptor. Science, **264**, 965–968.
24. GUIDOS C. J., WEISSMAN I. L. and ADKINS B. (1989): Developmental potential of $CD4^-8^-$ thymocytes: peripheral progeny include mature $CD4^-8^-$ T cells bearing αβ T cell receptor. J. Immunol., **142**, 3773–3780.
25. GUY-GRAND D., CERF-BENSUSSAN N., MALISSEN B., MALASSIS-SERIS M., BRIOTTET C. and VASSALLI P. (1991): Two gut intraepithelial $CD8^+$ lymphocyte populations with different T cell receptors. A role for the gut epithelium in T cell differentiation. J. Exp. Med., **173**, 471–481.
26. HASHIMOTO W., TAKADA K., ANZAI R., OGASAWARA K., SAKIHARA H., SUGIURA K., SEKI S. and KUMAGAI K. (1995): Cytotoxic NK1.1 Ag^+ αβ T cells with intermediate TCR induced in the liver of mice by IL-12. J. Immunol., **154**, 4333–4340.
27. HAYAKAWA S., SAITO S., NEMOTO N., CHISHIMA F., AKIYAMA K., SHIRAISHI H., HAYAKAWA J., SUZUKI M. K., FUJII K. T., ICHIJO M., SAKURAI I. and SATOH K. (1994): Expression of recombinase-activation genes (RAG-1 and -2) in human decidual mononuclear cells. J. Immunol., **153**, 4934–4939.
28. HEYBORN K. D., FU Y. X., KALATARADI H., REARDON C., ROARK C., EYSTER C., VOLLMER M., BORN W. and O'BRIEN R. L. (1993): Evidence that murine Vγ5 and γρ-TCR^+ lymphocytes are derived from a common distinct lineage. J. Immunol., **151**, 4523–4527.
29. HILBE W., EISTERER W., SCHMID C., STARZ I., SILLY H., DUBA C., LUDESCHER C. and THALER J. (1994): Bone marrow lymphocyte subsets in myelodysplastic syndromes. J. Clin. Pathol., **47**, 505–507.
30. HUANG L. and CRISPE I. N. (1992): Distinctive selection mechanisms govern the T cell receptor repertoire of peripheral $CD4^-$ $CD8^-$ α/β T cells. J. Exp. Med., **176**, 699–706.
31. IIAI T., WATANABE H., SEKI S., SUGIURA K., HIROKAWA K., UTSUYAMA M., TAKAHASHI-IWANAGA H., IWANAGA T., OHTEKI T. and ABO T. (1992): Ontogeny and development of extrathymic T cells in mouse liver. Immunology, **77**, 556–563.

32. IZQUIERDO M., BALBOA M. A., FERNANDEZ-RANADA J. M., FIGUERA A., TORRES A., IRIONDO A. and LOPEZ-BOTET M. (1990): Relation between the increase of circulating $CD3^+CD57^-$ lymphocytes and T cell dysfunction in recipients of bone marrow transplantation. Clin. Exp. Immmunol., **82**, 145–150.
33. JOYCE S., NEGISHI I., BOESTEANU A., DESILVA A. D., SHARMA P., CHORNEY M. J., LOH D. Y. and KAER L. V. (1996): Expansion of natural ($NK1^+$) T cells that express αβ T cell receptors in transporters associated with antigen presentation-1 null and thymus leukemia antigen positive mice. J. Exp. Med., **184**, 1579–1584.
34. KAWACHI Y., WATANABE H., MORODA T., HAGA M., IIAI T., HATAKEYAMA K. and ABO T. (1995): Self-reactive T cell clones in a restricted population of IL-2 receptor β^+ cells expressing intermediate levels of the T cell receptor in the liver and other immune organs. Eur. J. Immunol., **25**, 2272–2278.
35. KAWAMURA T., SEKI S., TAKEDA K., NARITA J., EBE Y., NAITO M., HIRAIDE H. and ABO T. (1999): Protective effect of $NK1.1^+$ T cells as well as NK cells against intraperitoneal tumors in mice. Cell. Immunol., **193**, 219–225.
36. KIKLY K. and DENNERT G. (1992): Evidence for extrathymic development of TNK cells. $NK1^+CD3^+$ cells responsible for acute marrow graft rejection are present in thymus-deficient mice. J. Immunol., **149**, 403–412.
37. KIMURA M., HANAWA H., WATANABE H., OGAWA M. and ABO T. (1995): Synchronous expansion of intermediate TCR cells in the liver and uterus during pregnancy. Cell. Immunol., **162**, 16–25.
38. KIMURA M., WATANABE H., OHTSUKA K., IIAI T., TSUCHIDA M., SATO S. and ABO T. (1993): Radioresistance of intermediate TCR cells and their localization in the body of mice revealed by irradiation. Microbiol. Immunol., **37**, 641–652.
39. KOSEKI H., ASANO H., INABA T., MIYASHITA N., MORIWAKI K., LINDAHL K. F., MIZUTANI Y., IMAI K. and TANIGUCHI M. (1991): Dominant expression of a distinctive $V14^+$ T-cell antigen receptor α chain in mice. Proc. Natl. Acad. Sci. USA, **88**, 7518–7522.
40. LACHAPELLE M. H., MIRON P., HEMMINGS R. and ROY D. C. (1996): Endometrial T, B, and NK cells in patients with recurrent spontaneous abortion. Altered profile and pregnancy outcome. J. Immunol. **156**, 4027–4034.
41. LEVITSKY H. I., GOLUMBEK P. T. and PARDOLL D. M. (1991): The fate of $CD4^-8^-$ T cell receptor-$\alpha\beta^+$ thymocytes. J. Immunol., **146**, 1113–1117.
42. LIN H., MOSMANN T. R., GUIBERT L., TUNTIPOPIPAT S. and WEGNANN T. G. (1993): Synthesis of T helper 2-type cytokines at the maternal-fetal interface. J. Immunol., **151**, 4562–4573.
43. MAKINO Y., YAMAGATA N., SASHO T., ADACHI Y., KANNO R., KOSEKI H., KANNO M. and TANIGUCHI M. (1993): Extrathymic development of Vα14 – positive T cells. J. Exp. Med., **177**, 1399–1408.
44. MARUYAMA S., TSUKAHARA A., SUZUKI S., TADA T., MINAGAWA M., WATANABE H., HATAKEYAMA K. and ABO T. (1999): Quick recovery in the generation of self-reactive $CD4^{low}$ NKT cells by an alternative intrathymic pathway when restored from acute thymic atrophy. Clin. Exp. Immunol., **117**, 587–595.
45. MIENO M., SUTO R., OBATA Y., UDONO H., TAKAHASHI T., SHIKU H. and NAKAYAMA E. (1991): $CD4^-8^-$ T cells receptor αβ T cell: generation of an *in vitro* major histocompatibility complex I specific cytotoxic T lymphocyte response and allogeneic tumor rejection. J. Exp. Med., **174**, 193–201.
46. MINAGAWA M., NARITA J., TADA T., MARUYAMA S., SHIMIZU T., BANNAI M., OYA H., HATAKEYAMA K. and ABO T. (1999): Mechanisms underlying immunologic states during pregnancy: possible association of the sympathetic nervous system. Cell. Immunol., **196**, 1–13.
47. MIYAJI C., WATANABE H., MINAGAWA M., TOMA H., NOHARA Y., NOZAKI H., SATO Y. and ABO T. (1997): Numerical and functional characteristics of lymphocyte subsets in centenarians. J. Clin. Immunol., **17**, 420–429.
48. MIYAJI C., WATANABE H., OSMAN Y., KUWANO Y. and ABO T. (1996): A comparison of proliferative response to IL-7 and expression of IL-7 receptors in intermediate TCR cells of the liver, spleen, and thymus. Cell. Immunol., **169**, 159–165.
49. MORODA T., IIAI T., KAWACHI Y., KAWAMURA T., HATAKEYAMA K. and ABO T. (1996): Restricted appearance of self-reactive clones into intermediate T cell receptor cells in neonatally thymectomized mice with autoimmune disease. Eur. J. Immunol., **26**, 3084–3091.
50. MORODA T., IIAI T., TSUKAHARA A., FUKUDA M., SUZUKI S., TADA T., HATAKEYAMA K. and ABO T. (1997): Association of granulocytes with ulcer formation in the stomach of rodents exposed to restraint stress. Biomed. Res., **18**, 423–437.
51. MORODA T., KAWACHI Y., IIAI T., TSUKAHARA A., SUZUKI S., TADA T., WATANABE H., HATAKEYAMA K. and ABO T. (1997): Self-reactive forbidden clones are confined to pathways of intermediate T cell receptor cell differentiation even under immunosuppressive conditions. Immunology, **91**, 88–94.
52. MOSLEY R. L., STYRE D. and KLEIN J. R. (1990): Differentiation and functional maturation of bone marrow-derived intestinal epithelial T cells expression membrane T cell receptor in athymic radiation chimeras. J. Immunol., **145**, 1369–1375.
53. MUSHA N., YOSHIDA Y., SUGAHARA S., YAMAGIWA S., KOYA T., WATANABE H., HATEKEYAMA K. and ABO T. (1998): Expansion of $CD56^+$NK T and γδT cells from cord blood of human neonates. Clin. Exp. Immmunol., **113**, 220–228.
54. NARITA J., MIYAJI C., WATANABE H., HONDA S., KOYA T., UMEZU H., USHIKI T., SUGAHARA S., KAWAMURA T., ARAKAWA M. and ABO T. (1998): Differentiation of forbidden T cell clones and granulocytes in the parenchymal space of the liver in mice treated with estrogen. Cell. Immunol., **185**, 1–13.
55. OHTEKI T., ABO T., SEKI T., KOBATA T., YAGITA H., OKUMURA K. and KUMAGAI K. (1991): Predominant appearance of γδT lymphocytes in the liver of mice after birth. Eur. J. Immunol., **21**, 1733–1740.
56. OHTEKI T., SEKI S., ABO T. and KUMAGAI K. (1990): Liver is a possible site for the proliferation of abnormal $CD3^+4^-8^-$ double-negative lymphocytes in autoimmune MRL-*lpr/lpr* mice. J. Exp. Med., **172**, 7–12.
57. OHTSUKA K., HASEGAWA K., YAMAGIWA S., SATO K., NAKAYAMA M., WATANABE H., SAKURA H. and ABO T. (1996): Intraepithelial lymphocytes in colon have similar properties to intraepithelial lymphocytes in small intestine and hepatic intermediate TCR cells. Digest. Dis. Sci., **41**, 902–911.
58. OHTSUKA K., IIAI T., WATANABE H., TANAKA T., MIYASAKA M., SATO K., ASAKURA H. and ABO T. (1994): Similarities and differences between extrathymic T cells residing in mouse liver and intestine. Cell. Immunol., **153**, 52–66.
59. OHTSUKA K., SATO K., WATANABE H., KIMURA M., ASAKURA H. and ABO T. (1995): Unique order of the lymphocyte subset induction in the liver and intestine of mice during *Listeria monocytogenes* infection. Cell. Immunol., **161**, 112–124.

60. OKADA T., IIAI T., KAWACHI Y., MORODA T., TAKII Y., HATAKEYAMA K. and ABO T. (1995): Origin of $CD57^+$ T cells which increase at tumour sites in patients with colorectal cancer. Clin. Exp. Immunol., **102**, 159–166.
61. OSMAN Y., WATANABE T., KAWACHI Y., SATO K., OHTSUKA K., WATANABE H., HASHIMOTO S., MORIYAMA Y., SHIBATA A. and ABO T. (1995): Intermediate TCR cells with self-reactive clones are effector cells which induce syngeneic graft-versus-host disease in mice. Cell. Immunol., **166**, 172–186.
62. PALATHUMPAT V., JONES D. S., HOLM B., WANG H., LIANG O. and STROBER S. (1992): Studies of $CD4^-CD8^-$ αβ bone marrow T cells with suppressor activity. J. Immunol., **148**, 373–380.
63. PAPIERNIK M. and PONTOUX C. (1990): *In vivo* and *in vitro* repertoire of $CD3^+CD4^-CD8^-$ thymocytes. Int. Immunol., **2**, 407–412.
64. PEARSE M., GALLAGHER P., WILSON A., WU L., FISICARO N., MILLER J. F. A. P., SCOLLAY R. and SHORTMAN K. (1988): Molecular characterization of T-cell antigen receptor expression by subsets of $CD4^-CD8^-$ murine thymocytes. Proc. Natl. Acad. Sci. USA, **85**, 6082–6086.
65. PRUD'HOMME G. J., BOCARRO D. C. and LUKE E. C. H. (1991): Clonal deletion and autoreactivity in extrathymic $CD4^-CD8^-$ (double negative) T cell receptor-α/β T cells. J. Immunol., **147**, 3314–3318.
66. REIMANN J., BELLAN A. and CONRADT P. (1988): Development autoreactive $L3T4^+$ T cells from double-negative ($L3T4^-$/$Ly\text{-}2^-$) Thy-1^+ spleen cells of normal mice. Eur. J. Immunol., **18**, 989–999.
67. SATO K., OHTSUKA K., HASEGAWA K., YAMAGIWA S., WATANABE H., ASAKURA H. and ABO T. (1995): Evidence for extrathymic generation of intermediate TCR cells in the liver revealed in thymectomized, irradiated mice subjected to bone marrow transplantation. J. Exp. Med., **182**, 759–767.
68. SATO K., OHTSUKA K., WATANABE H., ASAKURA H. and ABO T. (1993): Detailed characterizaiton of γδT cells within the organs in mice: Classification into three groups. Immunology, **80**, 380–387.
69. SEKI S., ABO T., MASUDA T., OHTEKI T., KANNO A., TAKEDA A., RIKIISHI H., NAGURA H. and KUMAGAI K. (1990): Identification of activated T cell receptor γδ lymphocytes in the liver of tumor-bearing hosts. J. Clin. Invest., **86**, 409–415.
70. SEKI S., ABO T., OHTEKI T., SUGIURA K. and KUMAGAI K. (1991): Unusual αβ T cells expanded in autoimmune *lpr* mice are probably a counterpart of normal T cells in the liver. J. Immunol., **147**, 1214–1221.
71. SHIMIZU T., SUGAHARA S., OYA H., MARUYAMA S., MINAGAWA M., BANNAI M., HATAKEYAMA K. and ABO T. (1999): The majority of lymphocytes in the bone marrow, thymus and extrathymic T cells in the liver are generated *in situ* from their own preexisting precursors. Microbiol. Immunol., **43**, 595–608.
72. SKINNER M. A., SAMBHARA S. R., BENVENISTE P. and MILLER R. G. (1992): Characterization of $\alpha\beta^+$ $CD4^-CD8^-$ CTL lines isolated from mixed lymphocyte cultures of adult mouse spleen cells. Cell. Immunol., **139**, 375–385.
73. SUDA T. and ZLOTNIK A. (1993): Origin, differentiation, and repertoire selection of $CD3^+CD4^-CD8^-$ thymocytes bearing either αβ or γδ T cell receptors. J. Immunol., **150**, 447–455.
74. SUGAHARA S., SHIMIZU T., YOSHIDA Y., AIBA T., YAMAGIWA S., ASAKURA H. and ABO T. (1999): Extrathymic derivation of gut lymphocytes in parabiotic mice. Immunology, **96**, 57–65.
75. SUMIDA T., TAKEI I. and TANIGUCHI M. (1984): Activation of acceptor-suppressor hybridoma with antigen specific suppressor T cell factor of two-chain type: requirement of the antigen- and the I- J-restricting specificity. J. Immunol., **133**, 1131–1136.
76. SUZUKI S., SUGAHARA S., SHIMIZU T., TADA T., MINAGAWA M., MARUYAMA S., WATANABE H., SAITO H., ISHIKAWA H., HATAKEYAMA K. and ABO T. (1998): Low level of mixing of partner cells seen in extrathymic T cells in the liver and intestine of parabiotic mice: Its biological implication. Eur. J. Immunol., **28**, 3719–3729.
77. TAKAHAMA Y., KOSUGI A. and SINGER A. (1991): Phenotype, ontogeny, and repertoire of $CD4^-CD8^-$ T cell receptor $\alpha\beta^+$ thymocytes. Variable influence of self-antigens of T cell receptor Vβ usage. J. Immunol., **146**, 1134–1141.
78. TAKII Y., HASHIMOTO S., IIAI T., WATANABE H., HATAKEYAMA K. and ABO T. (1994): Increase in the proportion of granulated $CD56^+$ T cells in patients with malignancy. Clin. Exp. Immunol., **97**, 522–527.
79. TANGRI S., BROSSAY L., BURDIN N., LEE D. J., CORR M. and KRONENBERG M. (1998): Presentation of peptide antigens by mouse CD1 requires endosomal localization and protein antigen processing. Proc. Natl. Acad. Sci. USA, **95**, 14314–14319.
80. TSUKAHARA A., SEKI S., IIAI T., MORODA T., WATANABE H., SUZUKI S., TADA T., HIRAIDE H., HATAKEYAMA K. and ABO T. (1997): Mouse liver T cells: their change with aging and in comparison with peripheral T cells. Hepatology, **26**, 301–309.
81. TSUKAHARA A., TADA T., SUZUKI S., IIAI T., MORODA T., MARUYAMA S., MINAGAWA M., MUSHA N., SHIMIZU T., HATAKEYAMA K. and ABO T. (1997): Adrenergic stimulation simultaneously induces the expansion of granulocytes and extrathymic T cells in mice. Biomed. Res., **18**, 237–246.
82. WATANABE H., MIYAJI C., KAWACHI Y., IIAI T., OHTSUKA K., IWANAGA T., TAKAHASHI-IWANAGA H. and ABO T. (1995): Relationships between intermediate TCR cells and $NK1.1^+$ T cells in various immune organs. $NK1.1^+$T cells are present within a population of intermediate TCR cells. J. Immunol., **155**, 2972–2983.
83. WATANABE H., MIYAJI C., SEKI S. and ABO T. (1996): c-kit^+ stem cells and thymocyte precursors in the livers of adult mice. J. Exp. Med., **184**, 687–693.
84. WATANABE H., OHTSUKA K., OBATA Y., IIAI T., KIMURA M., TAKAHASHI T., HIROKAWA K., UTSUYAMA M. and ABO T. (1993): Generalized expansion of extrathymic T cells in various immune organs of TL-transgenic mice. Biomed. Res., **14**, 273–288.
85. WATANABE T., KAWAMURA T., KAWAMURA H., HAGA M., SHIRAI K., WATANABE H., EGUCHI S. and ABO T. (1997): Intermediate T-cell receptor cells in mouse lung. Their effector function to induce pneumonitis in mice with autoimmune-like graft--versus-host disease. J. Immunol., **158**, 5805–5814.
86. WEERASINGHE A., KAWAMURA T., MORODA T., SEKI S., WATANABE H. and ABO T. (1998): Intermediate TCR cells can induce graft-versus-host disease after allogeneic bone marrow transplantation. Cell. Immunol., **185**, 14–29.
87. WILSON A., EWING T., OWENS T., SCOLLAY R. and SHORTMAN K. (1988): T cell antigen receptor expression by subsets of Ly--$2^-$$L3T4^-$ ($CD4^-CD8^-$) thymocytes. J. Immunol., **140**, 1470–1476.
88. YAMAGIWA S., SUGAHARA S., SHIMIZU T., IWANAGA T., YOSHIDA Y., HONDA S., WATANABE H., SUZUKI K., ASAKURA H. and

ABO T. (1998): The primary site of $CD4^{-}8^{-}B220^{+}$ αβT cells in *lpr* mice – the appendix in normal mice. J. Immunol., **160**, 2665–2674.

89. YAMAMOTO S., SATO Y., SHIMIZU T., HALDER R. C., OYA H., BANNAI M., HATAKEYAMA K. and ABO T. (1999): Consistent infiltration of thymus-derived T cells into the parenchymal space of the liver in normal mice. Hepatology, **30**, 705–713.
90. ZLOTNIK A., GODFREY D. I., FISCHER M. and SUDA T. (1992): Cytokine production by mature and immature $CD4^{-}CD8^{-}$ T cells. αβT cell $receptor^{+}$ $CD4^{-}CD8^{-}$ T cells produce IL-4. J. Immunol., **149**, 1211–1215.

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