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Positive selection of γδ T cells

Juan J. Lafaille, Werner Haas[1], Antonio Coutinho[2] and Susumu Tonegawa

The issue of T-cell repertoire selection has been addressed recently by several laboratories. While evidence has been provided for both negative and positive selection of CD4⁺ and CD8⁺ αβ T cells, the molecular basis of positive selection remains unclear. In this article Juan Lafaille and colleagues describe molecular features of γδ T-cell selection in the fetal thymus. These features were deduced from extensive junctional sequence data of γδ T-cell receptor genes in fetal thymocytes. Their data suggest the active participation of a self peptide in the positive selection of γδ T cells.

The rearrangement of variable gene segments generates an immense antigen receptor diversity (reviewed in Ref. 1). Antigen receptors, which are first expressed on immature lymphocytes, initially encounter host components. As a result of self recognition, the immature cell either dies (negative selection – first proposed by Lederberg[2]) or proceeds to differentiate into a mature lymphocyte (positive selection – first proposed by Jerne[3]).

Positive selection has been most clearly shown for the progenitors of the two major classes of αβ TCR-bearing cells, that is, MHC class I-restricted CD8⁺ T cells and MHC class II-restricted CD4⁺ T cells. The first evidence for positive selection came from the study of chimeric mice which consisted of MHC disparate hemopoietic and non-hemopoietic cells[4,5]. In such mice, T cells were shown to be selected in the thymus on the basis of their MHC-restriction specificity. Recently, the notion of positive selection has been extended in studies with αβ transgenic mice[6,7]. In these mice, the maturation of T-cell progenitors with predetermined (transgene encoded) αβ TCR was shown to depend solely on the intrathymic expression of the appropriate restriction element. The nominal antigen recognized by the transgenic TCR played no part in selection. The structural details of MHC restriction specificity are, at present, unknown but may be largely determined by those α and β chain regions that correspond to complementarity determining regions one and two (CDR1 and CDR2) of immunoglobulin chains[8]. This remains to be proved by X-ray crystallographic studies or by the comparison of sequences of TCR with known restriction specificity.

In the case of γδ T cells, while little is known about their specificity, some conclusions about selection can be drawn from γδ TCR sequence data. The sequences of many murine γδ TCR-encoding genes in cells from different peripheral tissues and from the thymus at various developmental stages have been determined[9–17]. Interestingly, a striking compartmentalization of γδ T cells, expressing different γ and δ gene segments, was found. γδ T cells in the fetal thymus, skin and certain mucosal epithelia use almost exclusively $V_\delta 1$ and $V_\gamma 5$ or $V_\gamma 6$; γδ T cells in the intestinal mucosa and mesenteric lymph nodes preferentially use $V_\gamma 7$; γδ T cells in other lymph nodes and spleen predominantly use $V_\gamma 4$ (reviewed in Ref. 18). On the basis of these findings it is possible that γδ T cells, like αβ T cells, differentiate into various subsets depending on their specificity. Indeed, our recent study[11] shows that the assumed dependence of γδ T-cell differentiation on the specificity of their TCR is probably true, at least for the two major γδ T-cell subsets which are generated in the fetal thymus. Each of these populations bears essentially

Howard Hughes Medical Institute at Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. [1]On leave of absence from Hoffmann La Roche, AG, Basel, Switzerland. [2]On leave of absence from Pasteur Institute, Paris, France.

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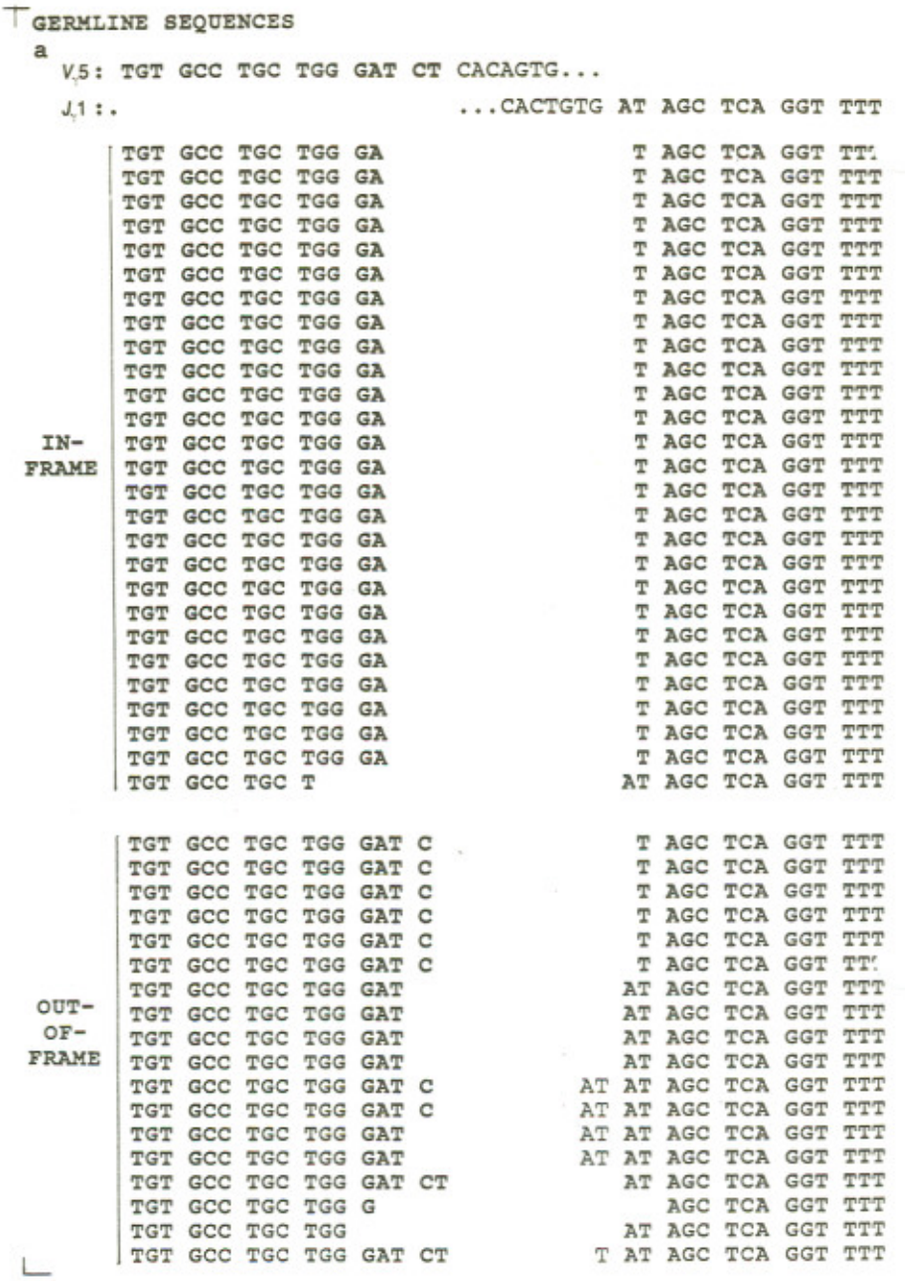
homogeneous TCR which consist of one particular δ chain ($V_\delta 1$-$D_\delta 2$-$J_\delta 2$) and one of two particular γ chains ($V_\gamma 5$-$J_\gamma 1$ or $V_\gamma 6$-$J_\gamma 1$)[11].

TCR diversity, like immunoglobulin diversity, is generated by the combination of different *V* and *J* or *V*, *D* and *J* gene segments. Additional diversity is generated at the junctions by exonucleolytic cleavage of some nucleotides from the joining ends as well as by template-free addition of nucleotides to these ends by the activity of a terminal transferase (reviewed in Ref. 1). The monospecificity found on the major fetal γδ T-cell subsets is therefore intriguing, since these rearrangements, even if restricted to $V_\delta 1$, $V_\gamma 5$ and $V_\gamma 6$, can be expected to generate some junctional diversity.

Junctional sequences that were determined in PCR-amplified thymocyte DNA show a striking homogeneity in the case of in-frame sequences but a clear, albeit limited, junctional diversity of out-of-frame sequences (Fig. 1). In $V_\gamma 6$-$J_\gamma 1$ out-of-frame junctions, one sequence also predominates, but this sequence is exceptional in that it can be generated in six different ways, making its frequent occurrence less surprising. In 18 hybridomas that were obtained by fusions of the thymoma BW5147 with fetal thymocytes, all but one of the in-frame junctions had the canonical sequences[11]. These findings indicate that, in the fetal thymus, there is a strong positive selection of T cells expressing either of the two canonical γδ TCR. The possibility that the sequence homogeneity in the in-frame junctions depends solely on the specificity of the recombinational machinery is highly unlikely because of the stochastic nature of the exonucleolytic activity involved. Another possibility is that the two canonical TCR represent the only two compatible pairs of all γ and δ chains generated in fetal thymocytes that can be expressed on the surface. However, we consider this possibility unlikely because the third complementarity determining region (CDR3), to which variations are restricted in these chains, should not be crucially involved in chain pairing[19]. We consider it much more likely that the selection of these cells is γδ TCR-ligand mediated. The ligands which select the major subsets of fetal γδ thymocytes must be self components that are expressed in the fetal thymus. Interestingly, the TCR regions that are subjected to positive selection in fetal γδ thymocytes correspond to the CDR3 regions of immunoglobulins. Thus, if these regions of γδ TCR are primarily involved in the interaction with antigen-derived peptides[8], a peptide must be crucial in the selection. In the case of $CD4^+$ and $CD8^+$ T cells, the active participation of self-antigen-derived peptides[20] has not been shown.



The nature of the self components selecting for the fetal γδ cells is unknown. However, it is possible that since the two canonical γδ TCR are identical in the region that corresponds to the CDR3, perhaps the two canonical γδ TCR of fetal thymocytes are specific for the same peptide. This peptide could be part of, or presented by, proteins encoded in the TL-region[21,22].

In conventional immune responses, an epitope selects not one, but a set of structurally related receptors. It is extraordinary, therefore, that only one TCR is selected in each fetal γδ T-cell subset. This seems to be accomplished by selecting from a relatively small receptor repertoire. In the fetal thymus, certain γ and δ gene segments preferentially rearrange, exonuclease activity is low, and termina transferase activity is absent[11]. Thus, both cellular and genetic mechanisms have evolved to ensure the generation of T cells with the canonical γδ TCR. These self-specific T cells must have beneficial functions within the

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GERMLINE SEQUENCES
b
   Vγ6 : TGT GCA TGC TGG GAT A CACTCTA...
   Jγ1 :                        ...CACTGTG AT AGC TCA GGT TTT

        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
IN-     | TGT GCA TGC TGG G                  AT AGT TCA GGT TTT
FRAME   | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA TGC TGG G                  AT AGC TCA GGT TTT
        | TGT GCA T                          AT AGC TCA GGT TTT

        | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
        | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
        | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
        | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
        | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
        | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
OUT-    | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
OF-     | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
FRAME   | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
        | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
        | TGT GCA TGC TGG GAT                AT AGC TCA GGT TTT
        | TGT GCA TGC TGG GAT A            T AT AGC TCA GGT TTT
        | TGT GCA TGC TGG GAT                  C TCA GGT TTT

GERMLINE SEQUENCES
c
   Vδ1 : TGT GGG TCA GAT AT
   Dδ1 :            CACTGTG GTGGCATATCA CACAGGT
   Dδ2 :                        CACCGTG ATCGGAGGGATACGAG CACAGTG
   Jδ2 :                                                TAACGTG C TCC TGG GAC

        | TGT GGG TCA GAT                   ATCGGAGGGA                G C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGA                G C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGA                G C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGA                G C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGA                G C TCC TGG GAC
IN-     | TGT GGG TCA GAT                   ATCGGAGGGA                G C TCC TGG GAC
FRAME   | TGT GGG TCA GAT                   ATCGGAGGGA                G C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGA                G C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGA                G C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGATACG              C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGATACG              C TCC TGG GAC
        | TGT GGG TCA GAT                     CGGAGGGATACGAG           C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGAT                C TCC TGG GAC

        | TGT GGG TCA GAT                   ATCGGAGGGATACGAG            C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGATACGAG            C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGATACGAG            C TCC TGG GAC
        | TGT GGG TCA GAT AT                ATCGGAGGGA                G C TCC TGG GAC
        | TGT GGG TCA GAT AT                ATCGGAGGGA                G C TCC TGG GAC
        | TGT GGG TCA G          ATATC GA    CGGAGGGATA                C TCC TGG GAC
OUT-    | TGT GGG TCA GAT                   ATCGGAGGGATA                 CC TGG GAC
OF-     | TGT GGG TCA G          ATATC         GGGATACGAG              C TCC TGG GAC
FRAME   | TGT GGG TCA GAT AT                ATCGGAGGGATACGAG            C TCC TGG GAC
        | TGT GGG TCA GAT A                   AGGGATACGAG              C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGATACGAG        A       GG GAC
        | TGT GGG TCA G                     ATCGGAGGGA                 TCC TGG GAC
        | TGT GGG TCA                         GATA                  G C TCC TGG GAC
        | TGT GGG TCA GAT                     CGGAGGGA                G C TCC TGG GAC
        | TGT GGG TCA GAT                   ATCGGAGGGATA              G C TCC TGG GAC
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Fig. 1. Junctional sequences of γδ genes from fetal and newborn thymocytes. *DNA from thymocytes obtained between day 14.5 of embryonic life and birth was subjected to polymerase chain reaction using $V_\gamma5$-$J_\gamma1$ (Fig. 1a), $V_\gamma6$-$J_\gamma1$ (Fig. 1b) and $V_\delta1$-$J_\delta2$ (Fig. 1c) pairs of primers, and the amplified DNA was cloned and sequenced[11]. A few nucleotides that can be assigned to either recombining gene segment were arbitrarily placed under one of the two gene segments. The out-of-frame sequences are more heterogeneous than the in-frame sequences. This seems not to be the case for the $V_\gamma6$-$J_\gamma1$ junctions. However, as outlined elsewhere[11] the 'canonical' out-of-frame $V_\gamma6$-$J_\gamma1$ junction is exceptional in that it can be generated in six different ways.*

thymus and/or at their peripheral destination[12,14,23].

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