Antibody and T-Cell Receptors

Susumu Tonegawa, MD

A VERTEBRATE is an attractive culture medium for viruses, bacteria, fungi, and metazoan parasites. It is therefore understandable that vertebrates have had to develop a body defense mechanism directed toward these pathogens for their emergence and survival. This defense mechanism is the immune system. The crucial event in mounting an immune response is the recognition of chemical markers that are present on infectious organisms and foreign bodies. The molecules entrusted to this task are the B-cell–synthesized antibodies and the antigen-specific T-cell receptors (TCRs). Antigens are numerous and structurally diverse. The fact that the body can produce antibodies against many artificially made chemicals and can discriminate among them attests to the vastness of the antigenic world. The antigen-binding specificity of an antibody or a TCR is determined by the amino acid sequence of the polypeptide chains that compose these proteins. The enormous diversity of antigens, therefore, translates directly to the equally enormous diversity of antibodies and TCRs. This brings us to the core of one of the most debated issues in immunology: Does an individual vertebrate inherit the whole set of genes necessary to code for *all* the seemingly infinite variety of antibody and TCR polypeptide chains? Many immunologists argued "yes." After all, as far as other proteins that had been studied were concerned, we inherit their genes. However, the vastness of the expected antibody diversity baffled some immunologists and led them to propose that some special genetic mechanisms may be involved in coding for these proteins.

See also pp 1834 and 1837.

In the early 1970s, when restriction enzymes and recombinant DNA technologies were introduced as tools to analyze specific eucaryotic genes, it became possible to critically test these ideas. It turned out that an individual does not inherit a single completed gene for antibody polypeptide chains.[1] Rather, we inherit pieces of genes, and these gene pieces are assembled by recombination in a large number of combinations during the differentiation of B lymphocytes. The exact ends of these gene segments vary from one recombination event to another, and in some cases a short nucleotide sequence is inserted at the joint in a template-independent fashion during the recombination. All of these somatic events occur before B cells meet antigens. Thus, our bodies prepare themselves with millions of B-cell clones, and the member cells of each clone express on their surface immunoglobulin (Ig) receptors of a unique specificity.

When an antigen enters the body, it will be screened by these virgin B cells with a variety of predetermined specificities. A small fraction of B cells carrying those Ig receptors that exhibit a sufficient affinity to the antigen will be selectively activated by the interaction with the antigen for propagation and differentiation. It is during this second antigen-dependent phase of B-cell differentiation that another important genetic mechanism, somatic hypermutation, is called for to "fine tune" the antigen-selected Ig molecules for tighter fitness. Those numerous B cells that fail to acquire affinity-improving mutations in the combining sites of their Ig will propagate relatively slowly and therefore will be diluted out. Thus, the antibodies secreted by the terminally differentiated B-lineage cells, called *plasma cells*, would usually have a much higher affinity than those present on the ancestral virgin B cells. The above is a brief summary of the generation of antibody diversity.

I now turn our attention to T cells. Unlike B cells, T cells recognize an antigen specifically in the molecular context of a self–major histocompatibility complex (MHC) gene product. The T-cell–made molecule, the TCR, responsible for this dual recognition of an antigen and an MHC had been elusive for many years. Because of the apparent exquisite specificity of T cells and the finding of the serologically common determinants (idiotype) on antibodies and TCRs, some immunologists proposed that Ig also is the chemical constituent of TCRs. However, preparation of effective sera and monoclonal antibodies that can immunoprecipitate T-cell clone–specific cell surface protein demonstrated that the TCR is a disulfide bond–linked heterodimer composed of α- and β-subunits of 40 to 45 kilodaltons each.

In the meantime, various attempts to clone TCR genes were made by molecular immunologists. The breakthrough along this line was made in 1983 by two independent groups, those of M. Davis and T. Mak, who employed what we now call a subtractive cDNA cloning method.[2,3] The clones isolated by them were for the β-subunit. Shortly afterward, Saito et al[4,5] also cloned a TCR β-

From the Center for Cancer Research, Massachusetts Institute of Technology, Cambridge.

Based on a lecture given at the presentation of the Albert Lasker Clinical Medical Research Award, New York, Nov 18, 1987.

Reprint requests to Center for Cancer Research, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02139 (Dr Tonegawa).

gene as well as the α-gene. These genes show a significant sequence homology to Ig genes, but are clearly distinct from them. T-cell receptor genes, like Ig genes, are split into V, J, C or V, D, J, C gene segments in the germline, and V-J and V-D-J assembly occurs in various combinations specifically during T-cell development. Thus, T and B cells use the common recombinational strategy to diversify the inherited genetic information for the respective protein. When it was realized earlier that the antigen specificity of a T cell is "restricted" by a self-MHC determinant, a question arose as to whether T cells carry one or two kinds of receptors. In the latter case, one receptor would be for antigen and the other for MHC. This question is now settled unequivocally for the former model by transfection experiments carried out using cloned TCR α- and β-genes.[6] How does a single receptor recognize determinants that are provided partially by an antigen and partially by an MHC molecule? Despite much progress made recently with respect to the nature of the antigens recognized by T cells and their interaction with MHC molecules, a final answer to this question is yet to come. It seems that the answer will only be obtained by a rigorous physicochemical analysis of the expected trimolecular complex composed of an antigen, an MHC product, and a TCR. Efforts toward this goal are being made in several laboratories.

I now turn to the third and final topic. During the search for the cDNA clones coding for the TCR α- and β-subunits, we discovered a third group of cDNA clones referred to as "gamma" whose gene is also specifically rearranged in the cytotoxic T-cell clone from which the subtractive cDNA clone library was made.[4,7] Besides the somatic rearrangement, the γ-gene shares a number of characteristics with the α- and β-genes. Thus, it is split into IgV-like, IgJ-like, and IgC-like gene segments in the germline; contains an exon coding for hydrophobic twenty-one residues reminiscent of a transmembrane peptide; and exhibits an overall nucleotide sequence homology of about 30% to both the α- and β-genes (which also are related to a similar extent). Existence of this third TCR-like rearranging gene was completely unexpected because the immunoprecipitation analysis of T-cell surface protein had not given any indication of the corresponding polypeptide chain. γ-cDNA isolated from the cytotoxic T-lymphocyte clone seemed to be derived from a perfectly translatable mRNA. However, it was soon shown that some cytotoxic T-lymphocyte and helper T-cell clones or hybridomas contain either no detectable γ-RNA or no inframe-rearranged γ-gene.[8-10] Thus, it was concluded that the expression of the γ-gene product is dispensable for the functioning of the conventional cytotoxic T lymphocytes and helper T cells. Another feature of the γ-gene is that its expressed repertoire is not very large. This was indicated by the fact that the Southern blot patterns of the DNA fragments containing the rearranged γ-genes were invariable when the genomic DNA isolated from several different T-cell clones were analyzed.[7] Furthermore, DNA isolated from a heterogeneous thymocyte population also gave the same pattern, with distinct, rearranged γ-gene bands. Subsequent cloning and sequencing analysis confirmed that the repertoire of γ-germline gene segments is limited. To date, only seven apparently functional V_γ gene segments (V_1 to V_7) and three pairs of J_γ-C_γ gene segments (J_1-C_1, J_2-C_2, and J_4-C_4) have been identified and characterized in mice. Furthermore, there seems to be a strong tendency for a given V gene segment to join only with the nearest J gene segment, thereby minimizing the "combinatorial diversity."

The first demonstration of the γ-protein came from the analysis of a subpopulation of human peripheral T cells. Brenner and colleagues[11] demonstrated that a small fraction of peripheral T cells from immunodeficient patients as well as healthy individuals express on their surface a heterodimer that is distinct from αβ. One of the subunits was shown to be encoded by the human equivalent of the mouse γ-gene using an antihuman γ-peptide. The other subunit, being distinct from the α-, β-, or γ-chain, was named *delta*. A similar heterodimer also was found on a population of human thymocytes.[12] The new heterodimer is associated with the monomorphic CD3 as the αβ-heterodimer is, but it is present on the surface of a population of thymocytes that show a surface phenotype distinct from that of the αβ-bearing cells: the former being primarily double negative (CD4 negative and CD8 negative) and the latter being single positive (CD4 positive and CD8 negative or CD4 negative and CD8 positive). Soon after the discovery of the γδ-bearing human T cells, cells of similar characteristics also were found in the double-negative populations of adult and fetal mouse thymocytes.[13-15] In our study along this line, we prepared both rabbit antisera and rat and mouse monoclonal antibodies directed against a mouse γ-polypeptide chain that had been synthesized in *Escherichia coli* using a bacterial expression vector. Others used antimouse CD3 monoclonal antibodies and anti–γ-antisera made with synthetic mouse γ-peptides. These studies revealed a number of interesting features of the γδ-receptors as well as the cells bearing them. First, as in humans, mouse γδ-heterodimers are bound relatively tightly to CD3, as evidenced by coimmunoprecipitation of the two proteins by a monoclonal anti–CD3 antibody from a digitonin extract of thymocytes. Second, in the case of the mouse, the γ- and δ-subunits are disulfide linked as the α- and β-subunits. Third, in the embryonic thymus, the appearance of the γδ-positive thymocytes precedes that of the αβ-positive cells: the former being detectable on day 14 but the latter not detectable until day 17. Fourth, there is developmental stage–dependent appearance of various kinds of γ-gene products: for instance, the $V_5J_1C_1$ and $V_4J_1C_1$ gene products appearing early and late, respectively, in thymic development.

The γδ-bearing cells also can be found in peripherals such as the spleen, lymph nodes, and blood, but they seem to be rare in mammals (<0.5% of peripheral T cells), suggesting that their primary role is in the thymus.[16] However, two observations indicate that this conclusion is premature. First, it was found that in chickens, 25% of Thy 1–positive, peripheral blood lymphocytes bear what are sure to be the chicken equivalent of mammalian γδ-heterodimers (M. D. Cooper, MD, and C. H. Chen, MD, personal communication, 1987). Second, in mice, Thy 1–positive dendritic epidermal cells are mostly double negative, and a considerable fraction of them carry γδ-heterodimers.[17,18] The rough estimate of the γδ-positive dendritic epidermal cells is 10 million per mouse (G. Stingl, MD, personal communication, 1987), a significant number compared with 100 million αβ-positive T cells in a whole mouse.

What is the function of γδ-bearing cells? The question can be divided into two parts: What is the ligand for the γδ-receptor? What effector function do the γδ-bearing cells have? Unfortunately, only limited information is available regarding these questions. In considering the first question, the extent of the structural diversity of γδ-receptors is an important issue. As already referred to previously herein, one of the earlier findings regarding the mouse γ-gene is that its germline repertoire is limited. This also seems to hold for humans.[19,20] However, the recent identification of J_δ and C_δ gene segments within the α-gene family and an appar-

ent overlap of the V_α and V_β gene segment pools suggest that delta diversity may be substantial.[21] In addition, the so-called junctional diversity applies to both the γ- and δ-chains. Thus, it is likely that the γδ-receptors would show considerable structural diversity that is clonally distributed. Structurally diverse receptors suggest structurally diverse ligands and, therefore, antigens and MHCs are two obvious candidates for ligands for the γδ-receptors. To test whether proteins encoded by MHC may play a role as the γδ–TCR ligand, we analyzed the effect of allo-MHC products on the stimulation of the γδ-positive cells in mixed lymphocyte cultures. In this analysis, we took advantage of the fact that in a certain strain of mice (Balb/c or B57 Black 6), some γ-chains are *N*-glycosylated while others (J_2C_2 encoded) are not, thereby allowing their distinction in sodium dodecylsulfate–polyacrylamide gel electrophoresis combined with glycanase treatment. When the γδ-heterodimers on unstimulated Balb.B (H-2^b) lymph node T cells were analyzed, we found that more than 90% of them are *N*-glycosylated and presumably encoded by J_1C_1-linked γ-genes. When these cells were cultured in the presence of roentgen ray–irradiated spleen cells from Balb.K (H-2^k) or B10.BR (also H-2^k) and the γδ-receptors on the Balb.K blast cells were analyzed, we found that more than 50% of them are the unglycosylated type. No such change of the γ-type was observed when the same responder cells were stimulated with Balb/c (H-2^d) spleen cells.[16]

A somewhat similar but much more extensive analysis of the role of allo-MHC as the target of the γδ-receptor was carried out by Bluestone and colleagues (personal communication, 1987). They prepared lines of γδ-bearing T cells from nude spleen T cells by stimulating them repeatedly with allo-MHC–positive spleen cells. These γδ-positive cells were shown to be cytotoxic to the target cells displaying a specific combination of MHC gene products. Studies using a variety of H-2 congenic and H-2 recombinant mouse strains suggest that the genes mapped to the right of H-2D are involved. This is an interesting and provoking observation because many class I–like genes mapped in the Qa and TL regions of the MHC are in search of a function for their gene products. It may be that the γδ-receptor has evolved to recognize partially or fully the class I–like glycoproteins that are distinct from those encoded by K, D, and L recognized by the αβ-receptors.

As to the effector function of γδ-bearing cells, the work of Bluestone and coworkers and earlier studies by others with human cells indicate that these cells have cytotoxic function.[22,23] In addition, some of the hybridomas derived from thymocytes and dendritic epidermal cells have the capacity to produce a lymphokine, interleukin 2 (A. Kruisbeek, MD, and E. M. Shevach, MD, personal communication, 1987).

In conclusion, the function of the γδ-bearing T cells is currently unknown. However, data accumulated to date permit some serious speculations. Perhaps the γδ–T cells have evolved to primarily protect epithelia that cover the external and internal surfaces of the body (C. A. Janeway Jr, MD, personal communication, 1987). These tissues are often the first to encounter infectious agents and therefore may have acquired, through evolution, specialized T cells to protect them. Few αβ–T cells are detected in epidermal tissues, suggesting a division of labor between the two types of T cells. There have been reports in the literature that gut-associated epithelia contain abundant Thy 1–positive cells.[24,25] It would be interesting to discover which types of TCRs, if any, are expressed on these and other epithelial T cells. A division of labor also may apply to the ligands: αβ-cells are reactive to or restricted by MHC K, D, and L gene products while γδ-cells are reactive to non-K, non-D, and non-L MHC gene products such as Qa and TLa.

Another interesting feature of the γδ-cells is their development. The existence of these cells in the spleen of nude mice suggests an extrathymic pathway of development. However, γδ-cells are relatively abundant in both fetal and adult thymi, and their intrathymic role should be considered. A key question is whether the intrathymic γδ-cells have any role in the development and repertoire selection of the αβ–T cells. We have no clue to answer this question but a number of studies currently being conducted in our laboratories and others are expected to help answer this and other questions concerning these new types of thymocytes and T cells whose existence was beyond anyone's imagination until just a year ago.

References

1. Tonegawa S: Somatic generation of antibody diversity. *Nature* 1983;302:575-581.
2. Hedrick SM, Nielsen EA, Kavaler J, et al: Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins. *Nature* 1984;308:153-158.
3. Yanagi Y, Yoshikai Y, Leggett K, et al: A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. *Nature* 1984;308:145-149.
4. Saito H, Kranz DM, Takagaki Y, et al: Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. *Nature* 1984;309:757-762.
5. Saito H, Kranz DM, Takagaki Y, et al: A third rearranged and expressed gene in a clone of cytotoxic T lymphocytes. *Nature* 1984;312:36-40.
6. Dembic Z, Haas W, Weiss S, et al: Transfer of specificity by murine α and β T-cell receptor genes. *Nature* 1986;320:232-238.
7. Kranz DM, Saito H, Heller M, et al: Limited diversity of the rearranged T-cell gamma gene. *Nature* 1985;313:752-755.
8. Reilly EB, Kranz DM, Tonegawa S, et al: A functional gamma gene formed from known gamma-gene segments is not necessary for antigen-specific responses of murine cytotoxic T lymphocytes. *Nature* 1986;321:878-880.
9. Rupp F, Frech G, Hengartner H, et al: No functional gamma-chain transcripts detected in an alloreactive cytotoxic T-cell clone. *Nature* 1986; 321:876-878.
10. Heilig J, Tonegawa S: T-cell gamma gene is allelically but not isotypically excluded and is not required in known functional T-cell subsets. *Proc Natl Acad Sci USA* 1987;84:8070-8074.
11. Brenner MB, McLean J, Dialynas DP, et al: Identification of a putative second T-cell receptor. *Nature* 1986;322:145-149.
12. Bank I, DePinho RA, Brenner MB, et al: A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes. *Nature* 1986;322:179-181.
13. Lew AM, Pardoll DM, Maloy WL, et al: Characterization of T cell receptor gamma chain expression in a subset of murine thymocytes. *Science* 1986;234:1401-1405.
14. Tonegawa S: Evolutionary aspects of immunoglobulin-related genes. *Chemica Scripta* 1986;26: 343-349.
15. Pardoll DM, Fowlkes BJ, Bluestone JA, et al: Differential expression of two distinct T-cell receptors during thymocyte development. *Nature* 1987;326:79-81.
16. Maeda K, Nakanishi N, Rogers BL, et al: Expression of the T-cell receptor γ-chain gene products on the surface of peripheral T cells and T-cell blasts generated by allogeneic mixed lymphocyte reaction. *Proc Natl Acad Sci USA* 1987;84:6536-6541.
17. Tschachler E, Schuler G, Hutterer J, et al: Expression of Thy-1 antigen by murine epidermal cells. *J Invest Dermatol* 1983;81:282-285.
18. Bergstresser PR, Tigelaar RE, Dees JW, et al: Thy-1 antigen-bearing dendritic cells populate murine epidermis. *J Invest Dermatol* 1983;81:286-288.
19. Quertermous T, Murre C, Dialynas D, et al: Human T-cell gamma chain genes: Organization, diversity, and rearrangement. *Science* 1985;231: 252-255.
20. LeFranc M-P, Forster A, Baer R, et al: Diversity and rearrangement of the human T cell rearranging gamma genes: Nine germ-line variable genes belonging to two subgroups. *Cell* 1986;45: 237-246.
21. Chien Y-H, Iwashima M, Kaplan K, et al: A new T-cell receptor gene located within the alpha locus and expressed early in T-cell differentiation. *Nature* 1987;327:677-682.
22. Borst J, Van de Griend RJ, van Oostveen JW, et al: A T-cell receptor γ/CD3 complex found on cloned functional lymphocytes. *Nature* 1987;325: 683-688.
23. Moingeon P, Jitsukawa S, Faure F, et al: A γ-chain complex forms a functional receptor on cloned human lymphocytes with natural killer-like activity. *Nature* 1987;325:723-726.
24. Ernst PB, Befus AD, Bienenstock J: Leukocytes in the intestinal epithelium: An unusual immunological compartment. *Immunol Today* 1985;6:50-54.
25. Klein JR: Ontogeny of the Thy-1−, Lyt-2+ murine intestinal intraepithelial lymphocyte: Characterization of a unique population of thymus-independent cytotoxic effector cells in the intestinal mucosa. *J Exp Med* 1986;164:309-314.