Antigen R

γδ T-cell receptors (ted, and γδ T cells are important mai mmu whereas B cells and αβ T cells, s hig polymorphic receptors, provide a nity. receptors of T and B lymphocytes are closely related. T belong to the immunoglobulin superfamily and composed of two disulfide-linked chains, which encoded by genes formed by rearrangement of vari (V), diversity (D), junctional (J) and constant (C) elements. Their antigen-binding site is formed by complementary determining region (CDR) loops, t formed by each chain. Two of the CDR loops are enco by V gene segments (CDR1 and CDR2) and one, the m polymorphic one, by recombination of V, (D) an segments (CDR3). B- and T-cell receptors *per se* have signalling capability, but are associated with invar chains that mediate signal transduction. By contrast, cells have two receptors, one activating and one inhibi (i.e. inhibits NK activation if the target cell expre adequate self major histocompatibility complex (M class I molecules

Secondary article

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cells. The final stage of B-cell differentiation results in cells that are either plasma cells, which produce large quantities of antibody and have a limited lifespan (about 1 month) or memory cells, which are small, long-lived cells that, upon antigen activation, can proliferate rapidly and differentiate into active B cells.

Structure of the B-cell receptor

B-cell receptors (BCRS) are monomeric immunoglobulins that, by alternative splicing, have C-terminal membrane-spanning regions and short cytoplasmic tails, thus membrane-bound and soluble immunoglobulins have the same variable domains, i.e. the same idiotypes. Surface and soluble immunoglobulins are composed of two heavy chains and two light chains of approximately 50 and 25 kDa, respectively. The two heavy chains are disulfide-linked and each heavy chain is linked to a light chain by a disulfide bond. Since the two heavy and light chains are identical, the antibody molecule has a twofold axis of symmetry. While there are only two types of light chains (κ and λ), there are five main heavy chain classes (IgM, IgD, IgG, IgA and IgE). Immature B cells express only monomeric IgM, whereas naive B cells co-express IgM and IgD, and memory B cells express IgG of different subclasses or IgE. Plasma cells do not express surface immunoglobulin.

Heavy and light chains are composed of constant (C) and variable (V) regions; those of the light chains are called V_L and C_L and those of the heavy chain V_H, C_{H1}, C_{H2} and C_{H3}. C_{H2} and C_{H3} together form the Fc portions that express isotype-specific differences. Enzymatic removal of the Fc portion results in Fab_2 fragments, which can be further cleaved into monomeric Fab fragments, that resemble TCRs. The structure of a Fab fragment of an IgG molecule is shown in (**Figure 1**). The CDRs, three from the heavy and three from the light chain, form the antigen-binding site. Affinity maturation of antibodies relies on

B-cell Receptor

The first stage of B-cell development in mammalians occurs in the bone marrow and includes rearrangement first of heavy, then of light chain of surface immunoglobulin, IgM. Immature B cells then migrate into the spleen and lymph organs, where they encounter foreign antigen. With the exception of T cell-independent antigens (e.g. B-cell mitogens or certain high molecular weight bacterial polysaccharides), further development of B cells including surface expression of IgD, production of soluble immunoglobulins, isotype switching and affinity maturation by somatic hypermutation) requires the help of T helper (T_H)

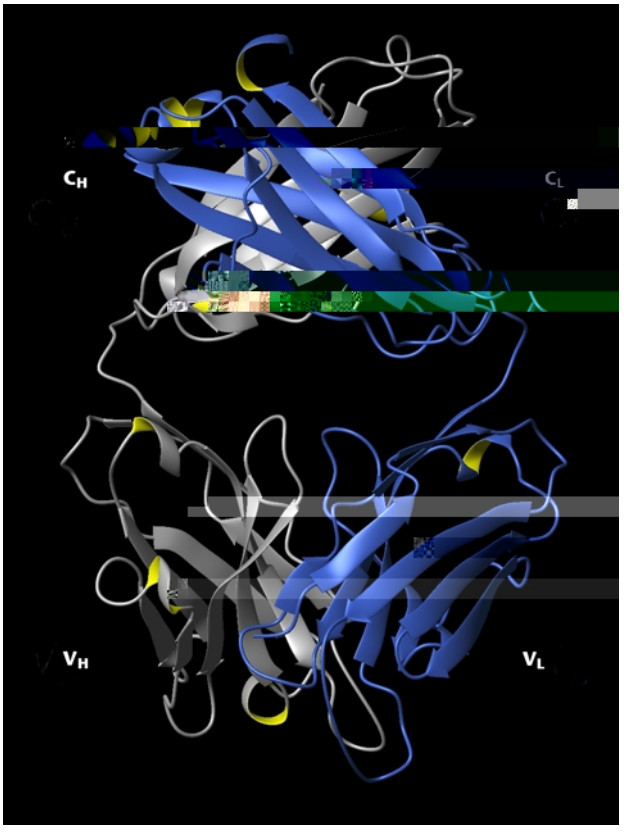

Figure 1 Fab fragment of human immunoglobulin (Ig)G monoclonal antibody CTM01. The IgG light chain is shown in blue and the heavy chain in grey. The antigen-binding site is formed by six CDR3 loops, three from V_L and three from V_H (Brookhaven PDB code 1AD9).

somatic hypermutation. The sites of the mutations are often distal to the combining site, and provide better complementarity.

B-cell coreceptor

The B-cell coreceptor consists of three molecules, CR2, the receptor for the activated complement component C3d, CD19 and TAPA-1 (CD81, target of an antiproliferative antibody) (Buhl and Cambier, 1997). While CR2 and CD19 are normal spanning proteins, TAPA-1 spans the membrane four times. Binding of ligands to CR2 lead to phosphorylation of CD19 and phosphorylated CD19 binds src family tyrosine kinases (e.g. lyn) and phosphatidylinositol 3-kinases. Co-ligation of the BCR and B-cell coreceptor allows CD19-associated tyrosine kinases to phosphorylate the BCR, thus augmenting its activation.

Antigen recognition by B cells

Surface expression of the BCR requires association with invariant Igα and Igβ heterodimers, which transduce BCR signals. Similar to the CD3 components of the TCR, the cytoplasmic tails of Igα and Igβ contain immunoreceptor tyrosine-based activation motifs (ITAMs), which upon BCR engagement are tyrosine phosphorylated by src kinases blk, lck, fyn and/or lyn. Syk, a cytosolic tyrosine kinase, binds to phosphorylated ITAMs and subsequently phosphorylates and activates Ras and phospholipase Cγ (PLCγ) (Birkeland and Monroe, 1997).

BCR signalling is induced by receptor crosslinking by antigen. Large antigens usually express multiple epitopes and thus crosslink the BCR by binding to several BCR molecules. The extreme case is that of T cell-independent antigens, i.e. high molecular weight polysaccharides with highly repetitive determinants, which cause extensive BCR crosslinking and hence strong BCR signalling. The strength of such BCR triggering is sufficient to drive B-cell differentiation to antibody production, independent of T-cell help. On the other hand, antibody responses to small organic molecules (haptens) necessitate that the hapten is conjugated on to a carrier protein or polysaccharide, as only then is BCR crosslinking possible. It is noteworthy that such hapten–glycoprotein conjugates can be formed *in vivo* by chemically reactive agents (e.g. certain drugs). If such antibody responses are of the IgE type, binding to basophils and mast cells, allergic reactions will occur.

Besides mediating signals, BCRs also promote efficient uptake of specific antigen. The internalized antigen is degraded in endosomal or lysosomal compartments and some of the resulting peptides bind to MHC class II molecules and are presented at the B-cell surface. This very effective antigen presentation to CD4 + T cells results in the induction and activation of T cells, which in turn can provide help to the B cells.

The majority of specific antibody responses are T-cell dependent. From very early developmental stages (early preB cell) B cells express CD40, which interacts with CD40 ligand (CD40L) on Th cells (Lederman *et al*., 1996). CD40 is a member of the tumour necrosis factor (TNF) receptor family of cytokine receptors and is analogous to Fas on cytotoxic T cells and to TNF receptor on macrophages. Binding of CD40 to CD40L helps to drive the resting B cell into the cell cycle. *In vitro*, B cells proliferate upon incubation with soluble CD40L and interleukin (IL)-4. IL-4 is also produced by activated Th cells and is secreted mainly at B-cell–T-cell contact sites, such that it acts primarily on the antigen-specific target B cell. IL-4 and CD40L are thought to synergize to promote clonal expansion before antibody production *in vivo*. Moreover CD40–CD40L interactions, together with specific cytokines, are required to induce isotype switching in B cells. Individuals with defective CD40 or CD40L produce only IgM antibodies and suffer from severe humoral immunodeficiency.

αβ T-cell Receptors

While B cells recognize native antigen, T cells recognize processed antigen (i.e. antigen-derived peptides bound to MHC molecules). CD4 + T cells recognize antigen in the context of MHC class II molecules and CD8 + cytotoxic T lymphocytes (CTLs) in the context of MHC class I molecules. The CD4 molecule restricts the interaction to class II molecules, while CD8 restricts the interaction to class I molecules. MHC class II molecules present exogenous antigens and class I molecules present endogenous antigens. Moreover, MHC class II, but not class I, molecules can bind superantigens. The interaction of MHC-bound superantigens with Vβ of TCRs results in polyclonal T-cell activation of T cells expressing particular Vβ.

Structure of T-cell receptors and T-cell receptor–ligand complexes

X-ray crystallographic studies of TCRs and TCR–ligand complexes (Garboczi *et al*., 1996; Garcia *et al*., 1996, 1998) showed that TCRs have an immunoglobulin-like structure, with some unique features: (i) the hinge region of the β chain is very rigid; (ii) TCR β chains have a solvent-exposed loop of 13 residues in Cβ; (iii) TCR Cα lacks a β-pleated sheet and thus has a poorly ordered structure; (iv) there is a strand switch in Vα, which results in a flattening of the outer surface of Vα. TCRs bind MHC class I–peptide complexes in a 'diagonal' orientation, in which the peptide runs diagonally between the two CDR3 loops, extending from CDR1α to CDR1β (**Figure 2**). This canonical orientation implies conserved atomic interactions between

dissociation of TCR–ligand complexes permits dynamic scanning of MHC–peptide complexes by TCRs on presenting cells.

T-cell activation and effector functions

The principal function of CD4 + effector T cells is to produce cytokines and to express surface effector molecules that belong to the tumour necrosis family, such as TNFα, CD40 ligand or Fas ligand. Upon activation naive CD4 + T cells proliferate and differentiate first into immature effector T cells (T_H0) and then either into inflammatory or T_H1 cells, or T_H2 cells. T_H1 cells mainly secrete IFNγ, GM-CSF and TNFα, which are macrophage-activating cytokines, but also various amounts of IL-3, leucotriene and IL-2. T_H2 cells mainly provide help to B cells and secrete the B-cell activation cytokines IL-4, IL-5 and IL-6, IL-3, GM-CSF, IL-10 and TGFβ. CD4 + T cells can also be cytotoxic by inducing apoptosis via Fas ligand expression.

Activated CD8 + CTLs kill target cells primarily by releasing perforin and granzymes, which in a synergistic manner induce target cell death. CTLs can also kill by expression of Fas ligand (FasL) on their surface, which binds to Fas on target cells and induces apoptosis. Activated CTLs also release cytokines, such as TNFα, interferon γ (IFNγ) and various interleukins. By binding to CD40 receptor on cells, TNFα can also induce apoptosis. Perforin-dependent killing is rapid (from a few minutes to a few hours) and can be elicited by a few MHC–peptide complexes on target cells. By contrast, cytokine production and FasL synthesis involve gene transcription, which requires sustained TCR signalling for extended periods of time. Fas-dependent cytolysis is slower than perforin-dependent cytolysis (6–18 h) and TNFα-mediated killing is slower still (after 16 h). In some cases Fas–FasL-dependent killing involves translocation of preformed, intracellular FasL to the cell surface or release of soluble FasL.

Altered peptide ligands

Modifications of antigenic peptides can affect functional T-cell responses in a diverse manner, for example they can alter the pattern of lymphokines produced (partial agonists) or antagonize T-cell responses to agonists (antagonists). For CD8 + T cells some partial agonists can elicit selective Fas-dependent cytotoxicity, which may play a role in maintaining homeostasis of lymphocytes. To explain aberrant T-cell function two basic concepts have been put forward: (1) the kinetic proofreading concept, which suggests that a short TCR engagement results in incomplete TCR signalling; and (2) the conformational model, which suggests that epitope modifications induce conformational changes in the TCR that qualitatively alter TCR signalling.

γδ T-cell receptors

γδ TCRs are in many respects similar to αβ TCRs and may by derived from a common progenitor. The δ chain resembles the α chain, and the γ chain resembles the β chain in composition and structure. γδ T cells constitute only 1–3% of CD3 + T cells. However, in epithelial tissue, especially in the small intestine and the epidermis, most of the T cells belong to the γδ lineage. The TCRs of these epithelial γδ T cells exhibit limited diversity, and most respond to the same antigens. Although the precise function of these cells is enigmatic, it seems that their contribution to host defence against infection consists more of innate than of adaptive immunity. Thus γδ T cells seem to recognize antigens common to a large number of unrelated pathogens, rather than distinct epitopes characteristic for individual organisms or species (Welsh *et al*., 1997).

Structure of γδ T-cell receptors

γδ TCRs are formed by rearrangement of V, (D), J and C gene elements, which precedes the one of αβ TCR genes (Chien *et al*., 1996). Remarkably, the gene complex encoding the δ chain is located between the ones of Vα and Jα segments, so that rearrangements at the α and δ loci are mutually exclusive. A rearranged δ chain comprises the Vδ, Dδ, Jδ and Cδ regions, and a rearranged γ chain the Vγ, Jγ and Cγ segments. Importantly, there are many fewer Vγ and Vδ gene segments than at the TCR Vα and Vβ loci. In humans there are only four Vδ, three Jδ, three Dδ and one Cδ, and 12 Vγ and two Cγ, each with its own Jγ segment. In the mouse the organization of the γ genes is more complex and there are three clusters of γ genes, each containing Vγ, Jγ and Cγ segments. The main diversity of γδ TCRs is in the junctional region, mainly because during δ chain rearrangement both D segments can be used in the same gene, which greatly increases the junctional variability.

γδ TCRs are expressed on the cell surface and are associated with CD3 and mediate TCR signalling in much the same way as αβ TCRs. The two chains are often, but not always, linked by a disulfide bridge between Cγ and Cδ. At present the three-dimensional structure of only one human Vδ domain is known (Li *et al*., 1998). Interestingly, this structure shows that the framework structure of Vδ resembles that of IgV_H more closely than that of Vα, Vβ or V_L, whereas the relative positions and conformations of its CDR1 and CDR2 share features of both Vα and V_H. Thus γδ TCRs seem to be structurally distinct from αβ TCRs. Together with the observation that the CDR3 length distribution of TCR δ chains is similar to that of immunoglobulin heavy chains, this suggests that γδ TCRs may bind antigen similarly to antibodies.

Antigen recognition by γδ T-cell receptors

Much of the antigen recognition by γδ T cells, the nature of the antigens and the presenting molecules, if any, are far from fully elucidated. γδ TCRs appear to recognize proteins directly, without antigen processing, and to recognize MHC molecules independently of bound peptide. Moreover, small phosphate-containing nonpeptide compounds found in various microorganisms and parasites have also been identified as ligands for certain γδ T cells (Morita *et al*., 1996). Conceivably γδ T cells recognize alterations of epithelial cells infected with any agent, such as expression of stress or heat shock proteins. Equally they recognize MHC class IB molecules that are expressed when epithelial cells are infected. These are specialized MHC class I-like molecules, which contain β_2-microglobulin and have a tissue-specific distribution. Their expression is under different regulatory control to MHC class I molecules, and in some cases they are induced in response to cellular stress (e.g. heat shock or certain infections). In the mouse, one of these molecules is H2-M3, which can present peptides with N-terminal *N*-formylmethionine, which is interesting because bacteria initiate protein synthesis with this residue. Other MHC class IB-like molecules are located in the Qa-Tla region (e.g T18) and can also be recognized by γδ T cells. Another potential ligand for γδ T cells is the CD1 molecule, the genes for which map outside the MHC region and which bind and present mycolic acid, a mycobacterial membrane component.

Biology of γδ T cells

Mature γδ T cells express many cell surface markers found on αβ T cells. They differ in that they are almost exclusively CD4 − and CD8 − . When activated, γδ T cells secrete the same cytokines as αβ T cells, although most human clones secrete low levels of IL-2 and hence their growth is more dependent on exogenous IL-2. During ontogeny of the lymphoid system, the γδ T cells are the first mature lymphocytes to appear during development of the embryo, at around day 14, thus before the development of αβ thymocytes. In the mouse, the γδ T cells are found predominantly in epithelium and less frequently in blood, whereas in humans they are also common in blood. The findings that γδ T cells are preferentially located in epithelium, proliferate during infection and that some of them are activated by mycobacterial components strongly suggest a significant role in early protective immunity. Since γδ T cells utilize receptors encoded by rearranging genes, this immunity may be seen as an interface between innate and adaptive immunity.

Natural Killer Cells

NK cells are granular lymphocytes which represent 5–14% of peripheral blood mononuclear cells. They are effector lymphocytes, contributing to early host defence against viral (e.g. herpes), and bacterial (e.g. *Listeria monoc togenes*) infections and lymphoid tumours by means of cytotoxicity and cytokine secretion (Trinchieri, 1989). NK cells can utilize either antibody-dependent cell-mediated cytotoxicity (ADCC) towards antibody-coated target cells or 'natural' cytotoxicity, which is regulated by a balance between ill-defined activating (i.e. killer cell activating receptor (KAR)) and MHC class I-specific inhibitory (killer inhibitory receptor (KIR)) receptors. Both receptors are expressed differently on different subsets of NK cells, which provides some variability in the structures that can be recognized by NK cells (Lanier, 1998).

Natural killer cell function

Although NK cells from uninfected organisms can kill sensitive targets, this activity is increased up to 100-fold in the presence of IFNα, IFNβ or IL-12, a monokine produced early in many infections. IL-12 in synergism with TNFα also activates NK cells to produce large amounts of IFNγ. This IFNγ production precedes that by T cells and plays a crucial role in controlling infection at an early stage. For example, severe combined immunodeficient mice, which lack T and B cells, can resist infection with *L. monoc togenes* this way.

ADCC by NK cells requires antibodies that bind cell-associated antigens such as viral or bacterial proteins. These antibodies bind to FcγRIII on NK cells, which recognize mainly IgG1 and IgG3. The resulting cross-linking of FcR elicits perforin–granzyme-dependent cytotoxicity, similar to CD8 + CTLs.

While NK cells play an important role in innate immunity, the fact that their KIRs recognize mainly MHC class I molecules, their function complements that of CD8 + CTLs as they eliminate cells that lack MHC class I expression, such as certain tumour cells or cells infected with viruses that have impaired MHC class I expression.

Natural killer cell activating receptors

The main NK cell receptor involved in activating natural cytotoxicity is NKR-P1. This receptor has the characteristics of a type C lectin and recognizes a wide variety of carbohydrate ligands, especially sulfated proteoglycans, constituents of the extracellular matrix of many cell types. However, depending on the state of activation and the availability of the relevant ligand on targe55;I;1plr2HSS[rN2H;0f

Moreover, subsets of NK cells express at their surface activating isoforms of inhibitory receptors for MHC class I molecules such as KARs, KIR counterparts such as CD94/NKG2 heterodimers (CD94/NKG2C) in humans, and Ly49 molecules (Ly49D and H) in mice (Vely and Vivier, 1997). These activating molecules share high homology (around 90%) in the extracellular portion with their inhibitory counterparts, but exhibit important differences in the transmembrane and cytoplasmic portions: the presence of a charged amino acid residue in the transmembrane domain and the lack of an immunoreceptor tyrosine-based inhibitory motif (ITIM). The activating receptors are associated with the ITAM-containing polypeptide killer activating receptor-associated protein (KARAP or DAP-12), expressed as a homodimer and responsible for transducing activating signals upon receptor crosslinking.

Although the structure and the mechanisms by which activating NKRs transduce stimulatory signals have been elucidated in some detail, the biological function of these receptors is still enigmatic. For example, it is not clear what role activating receptors play during NK cell maturation or whether cooperation exists between activating and inhibitory NKRs (i.e. whether, upon interaction with their ligand, the activating receptors recruit the kinases responsible for phosphorylation of the ITIM, thus enhancing the inhibitory activity of the ITIM-bearing receptors).

Natural killer cell inhibitory receptors

The KIRs specific for MHC class I molecules belong to the immunoglobulin superfamily (IgSF) or to the C-type lectin superfamily in humans; in the mouse only lectin-like receptors have been described (Lanier, 1998). The IgSF KIRs belong to a multigenic and multiallelic family encoded by genes of chromosome 19q13.4. They have two or three extracellular immunoglobulin-like domains, a transmembrane portion and a long intracellular domain containing two ITIMS. The integrity of ITIM sequences is necessary for the recruitment of cytoplasmic effector molecules involved in the inhibition of NK cells, namely the protein tyrosine phosphatases SHP-1 and SHP-2. Receptors of the KIR family with two immunoglobulin domains recognize human leucocyte antigen (HLA)-C molecules (e.g. CD158a interacts with HLA-Cw4; CD158b with HLA-Cw3), whereas the three immunoglobulin domain KIR NKB1/p70 molecule is specific for HLA-B alleles, and p140 interacts with certain HLA-A alleles (e.g. HLA-A3 and HLA A11).

The structure of a two-domain KIR is shown in (

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Further Reading

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