REVIEW ARTICLE

Cellular signalling mechanisms in B lymphocytes

William CUSHLEY and Margaret M. HARNETT
Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

INTRODUCTION

The B lymphocyte is the principal cellular mediator of the specific humoral immune response to infection. Activation of B cells occurs on selection of the appropriate clones by antigen leading to B cell proliferation and differentiation into antigen-specific, antibody-secreting plasma cells. After infection, the primed B lymphocyte serves as the cellular repository of specific immunological memory. The induction of antibody-secreting plasma cells and memory B cells is a complex process, integrating signals generated by a number of immunoregulatory receptors on several cell types, often in specialized environments: at each stage of development or differentiation, the fate of the B lymphocyte is determined not only by antigen but also by an array of soluble factors, including cytokines. In addition, signals generated by cell–cell contact and mediated by groups of complementary cell surface adhesion molecules also play a critical role in B cell development.

The B lymphocyte therefore provides an excellent model system in which to study not only cellular signalling processes but also cross-talk mechanisms by which distinct cell surface receptor-directed signal transduction pathways interact. This Review aims to detail the known structural and molecular pharmacological data relating to B cell antigen and cytokine receptors and, whilst identifying areas of current controversy, to set this molecular data in the context of the cell biology of B lymphocyte differentiation.

B LYMPHOCYTE DIFFERENTIATION

Antigen-independent differentiation

B lymphocytes arise from pluripotent stem cells in the bone marrow via a number of defined precursor B cell phenotypes (Figure 1). Once B cells emerge into the periphery, selection can lead to (i) cellular anergy and/or programmed cell death, (ii) activation, proliferation and differentiation into high rate antibody-secreting plasma cells, or (iii) differentiation to memory B lymphocytes. The complex, multi-stage differentiation of normal B lymphocytes can be conveniently considered as divisible into two phases; antigen-independent and antigen-dependent (reviewed by Rolink and Melchers, 1991). The antigen-independent phase of differentiation occurs in the bone marrow, is dependent upon growth factors derived from stromal cells, and is concerned with providing the B cell with a functional cell surface receptor for antigen, [i.e., with a membrane immunoglobulin (mIg) molecule capable of binding an antigen]. The Clonal Selection Theory (Burnet, 1959) dictates that each B lymphocyte expresses a receptor of a single antigen specificity; that is, antigen receptors are clonally distributed. The antigen-independent phase of B cell maturation proceeds via a series of stochastic rearrangements of the immunoglobulin heavy and light chain genes (Tonegawa, 1983), with the *Igh* locus being rearranged first followed, in order, by *Igk* and *Igl* (Coleclough et al., 1981). Productive rearrangement of one of the two alleles at each locus inhibits recombination at the other allele, thus providing a molecular explanation for allelic exclusion of antigen receptors. Successful rearrangement of the heavy chain gene followed by that of one of the two light chain genes results in stable expression of IgM at the B cell surface, and the B lymphocyte can now be thought of as entering the antigen-dependent phase of B cell development.

Antigen-dependent differentiation

The mIgM$^+$ "immature" B cell is the first B cell which has the opportunity to respond to challenge with antigen. The great weight of available data, mostly from B cell lymphomas and transgenic mouse models, suggests that stimulation of the antigen receptor on immature B cells results in either clonal unresponsiveness (anergy) or in deletion of the clone (Scott et al., 1987; Goodnow et al., 1988; Nemazee and Bürki, 1989; Ales-Martinez et al., 1991). In the latter case, clonal deletion is accomplished by driving the antigen-stimulated B cells into apoptosis, or programmed cell death (Hasbold and Klaus, 1990). The biological significance of clonal deletion or anergy of immature mIgM$^+$ B cells is that it allows for removal of those B cells which possess antigen receptors which bind to self tissue components. If activated, such B cells would secrete self-reactive antibodies, potentially leading to autoimmune disease.

The immature IgM$^+$ B cell next develops to express mIgD; the mIgM$^+$/mIgD^{++} B cell can be regarded as a "mature" B lymphocyte. The mature B cell reacts positively to ligation of the antigen receptor, but in the case of a thymus-dependent antigen (which includes the majority of protein antigens), the precise nature of the response is shaped by T cell-derived cytokines. The first possibility is for the antigen-activated B cell to become a high rate IgM antibody-secreting plasma cell, which expresses essentially no mIg and whose energies are devoted to production of the secretory form of IgM. Alternatively, the antigen-activated cell can undergo isotype switch, V region somatic mutation and emerge as a memory B cell, a series of events manifest in the plasma of the host as a more rapid response to re-challenge with the same antigen together with appearance of higher affinity antibodies of, for example, the IgG class.

Abbreviations used: mAb, monoclonal antibody; AP-1, activated protein-1; CD, cluster of differentiation; EGF, epidermal growth factor; FcR, Fc receptor; FDC, follicular dendritic cell; GH, growth hormone; GM-CSF, granulocyte/monocyte colony-stimulating factor; GPI, glycophosphatidylinositol; GTPγS, guanosine 5-[γ-thio]triphosphate; HRS, haematopoietin receptor superfamily; ICAM-1, intercellular adhesion molecule-1; mIg, membrane immunoglobulin; IFN, interferon; IL, interleukin; InsP_3, inositol trisphosphate; LFA, leukocyte functional antigen; MAP, mitogen-activated protein (kinase); MHC, major histocompatibility complex; NF-BRE, nuclear factor–B cell response element; NF-κB, nuclear factor κB; PDGF, platelet-derived growth factor; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PTK, protein tyrosine kinase; PTPase, protein tyrosine phosphate phosphatase; TcR, T cell receptor.

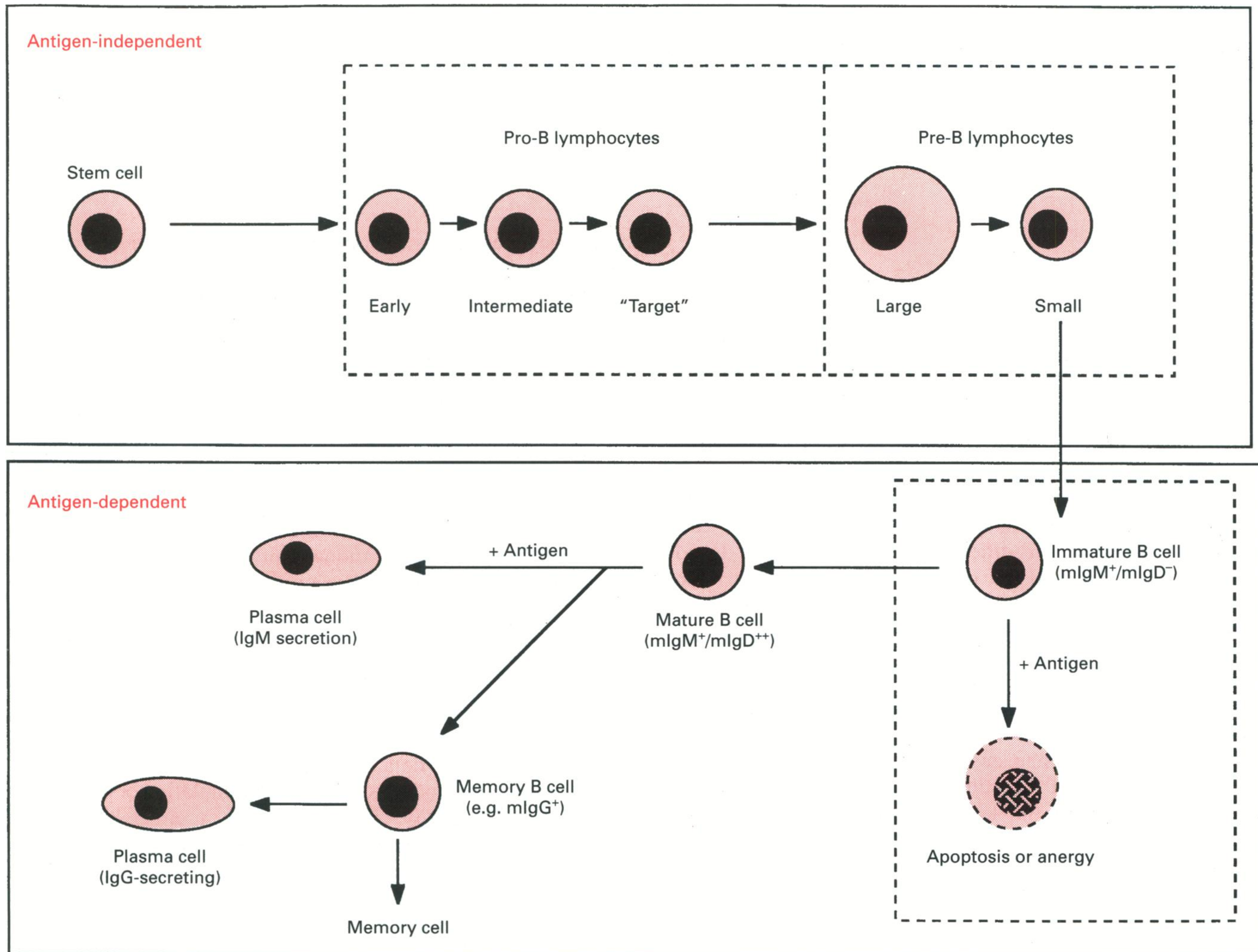

Figure 1 The B lymphocyte differentiation pathway

B lymphocyte differentiation is divisible into two phases, with antigen-independent steps which occur in the mammalian bone marrow and the antigen-dependent phase which takes place in the periphery (i.e. secondary lymphoid organs). The pathway illustrated is based on data from murine models. In the antigen-independent phase, the *Early* pro-B cell is $CD45^-$ but possesses a functionally rearranged heavy chain locus and is the first B cell precursor to express the *mb*-1 protein (despite being mIg^-). This cell also expresses the surrogate light chains $V_{pre\text{-}B}$ and λ_5 which are important in allelic exclusion and are present in all B cell precursor cells. The *Intermediate* pro-B cell is the first to express the CD45 antigen, and the *Target* pro-B cell is so called because it appears to be the target cell for the Abelson murine leukaemia virus. Pre-B cells are distinguished on the basis of size, with large cells being dividing cells. The small pre-B cells express cytoplasmic μ chains and are non-dividing cells. Each differentiation step from the Early pro-B cell to the small pre-B cell involves one round of division; this means that the productively rearranged VDJ gene in the early pro-B cell is found in 16 daughter small pre-B cells prior to light chain gene re-arrangement. Functional light chain rearrangement leads to expression of mIgM and the B cell is now reactive to antigen and enters the antigen-dependent phase of differentiation. Contact of an immature ($mIgD^-$) B cell with antigen leads to anergy or apoptosis of that cell, while a mature B cell ($mIgM^+/mIgD^{++}$) will respond to antigen by differentiating to a plasma cell or memory B cell depending upon the nature of T cell help available.

Cellular events in generation of memory B cells

The cellular events which give rise to memory B cells, the follicular reaction and germinal centre formation, have been the subject of study for many years and have recently been reviewed (Liu et al., 1992). Follicles are found in secondary lymphoid tissues (e.g. tonsil, spleen, lymph nodes) and consist of a network of follicular dendritic cells (FDCs) and, in the case of follicles where an antigen-driven response to a T-cell-dependent antigen is occurring, large numbers of lymphoblasts. The key property of FDCs is that they "fix" antigen on their cell surface in an unprocessed form in immune complexes which can later be recognized by newly-formed memory B lymphocytes. Germinal centre formation is a characteristic feature of a secondary immune response to antigen and the initial step in this process is the exponential growth of mIg^+ B cell blasts within the follicle. Once the FDC network is filled, a polarization of cells within the follicle occurs which gives rise to the characteristic histological appearance of a germinal centre (Figure 2). The B cell blasts move to one edge of the germinal centre, called the dark zone, lose expression of mIg and continue to proliferate; these cells are now referred to as centroblasts. The proliferating mIg^- centroblasts give rise to centrocytes which are non-dividing B cells which express mIg of isotypes other than IgM. The centrocytes move into the light zone of the germinal centre which possesses a dense network of FDCs, in contrast to the finer FDC network

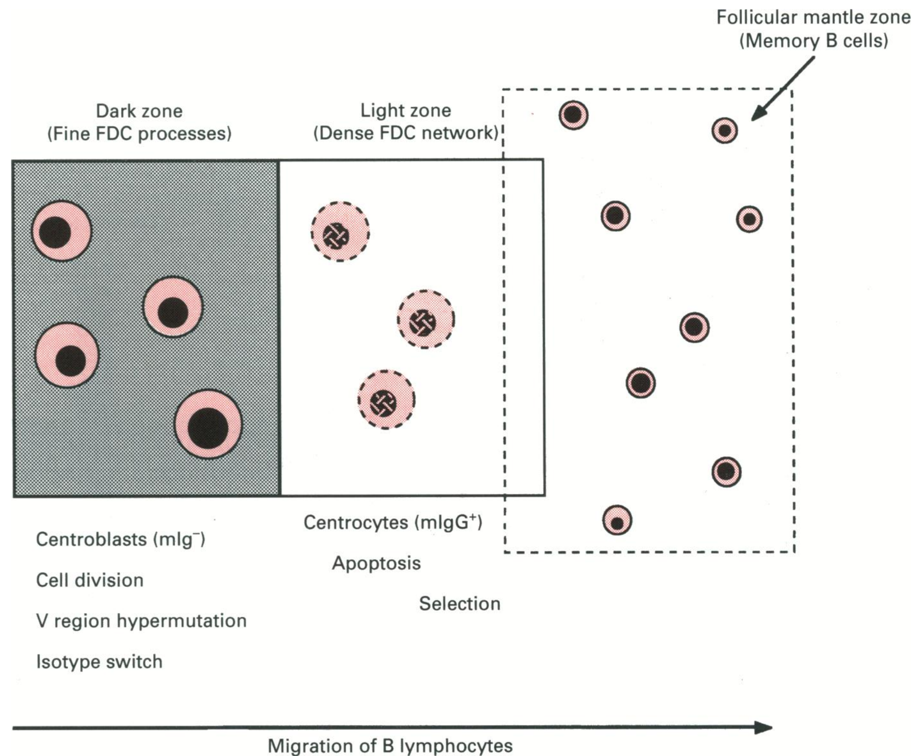

Figure 2 Cellular events in the generation of B cell memory

Memory B cells arise in germinal centres in secondary lymphoid organs. Antigen-activated B cells are driven to proliferate, move to the dark zone of the germinal centre and cease mIgM/mIgD expression; at the genetic level V region genes undergo somatic hypermutation, and the pattern of C region gene expression changes from μ and δ to, for example, Cγ. The centroblasts in the dark zone give rise to the centrocytes, smaller non-dividing B cells expressing mIgG (for example), which migrate to the light zone where they encounter antigen fixed as immune complexes on follicular dendritic cells (FDCs) and CD40 (the division of the light zone into basal and apical regions is not shown on the Figure). Centrocytes which have (for example) mIgG receptor for antigen fixed on the FDCs are selected and migrate to the follicular mantle zone as memory B cells.

processes found in the dark zone, and shows evidence of massive cell death by apoptosis. The light zone is divisible into two areas, the basal light zone adjacent to the dark zone and the apical light zone closer to the follicular mantle. Surviving memory cells emerging from the light zone reside in the follicular mantle zone.

In molecular terms, it seems likely that the mIg$^-$ centroblasts undergo heavy chain isotype switch and V gene somatic hypermutation to give rise to the non-proliferating centrocytes which express mIg of isotypes other than mIgM and mIgD. The centrocytes are now programmed to undergo apoptosis and die rapidly unless actively rescued from this fate. *In vitro* studies of human tonsillar centrocytic cells indicate that two signals enable the centrocyte to escape apoptosis; one is generated by the cross-linking of the antigen receptors (*in vivo*, presumably by antigen fixed on the surface of FDCs), whilst the other is provided by ligation of the CD40 antigen on the centrocyte cell surface (Liu et al., 1989). This rescue of centrocytes from apoptosis appears to occur in the basal light zone. Thus, newly-generated centrocytes have the opportunity to interrogate their somatically mutated and isotype-switched antigen receptors with the original antigen in these complexes and, if specific binding occurs, the cells are rescued from apoptosis. The main consequences of this process are that redundant specificities are not selected and only the highest affinity clones are rescued if antigen concentrations are limiting.

Further centrocyte maturation occurs in the apical light zone (Liu et al., 1992) where the cells can be induced to become either non-cycling memory cells via interaction with CD40 ligand (Liu et al., 1991a) or can be driven to differentiate to plasmablast-type cells following stimulation with soluble CD23 and IL-1α (Liu et al., 1991a, 1992) or with IL-2 (Holder et al, 1992). This finding correlates well with the observation that apical light zone FDCs produce large amounts of CD23 and soluble CD23 (sCD23), the latter presumably acting upon the centrocytes via a paracrine mechanism. The signals delivered to the centrocyte as a consequence of encounter with each of these ligands and the importance of each signal in determining the survival and differentiation of the centrocyte remain to be elucidated.

The antigen receptor: death, life and suicide

The above brief account of B cell differentiation indicates that antigen plays a critical role in determining the fate of the B cell in the antigen-dependent phase of B cell development. Thus, immature B cells are clonally deleted, or at least functionally tolerised, if their antigen receptor is stimulated. This is in striking contrast to the situation with mature B cells or the immediate precursors of memory cells, the centrocytes, which respond to antigen stimulation by activation or rescue from cell suicide, respectively. However, it appears that, at least in models of immature and mature B cells, the signals transduced via the B cell antigen receptor are very similar and involve protein tyrosine kinase (PTK) activation, phospholipase C (PLC)-mediated hydrolysis of the inositol phospholipid phosphatidylinositol 4,5-bisphosphate (PtdInsP_2) and elevation of intracellular Ca^{2+} concentration (Monroe and Cambier, 1983 ; Bijsterbosch et al.,

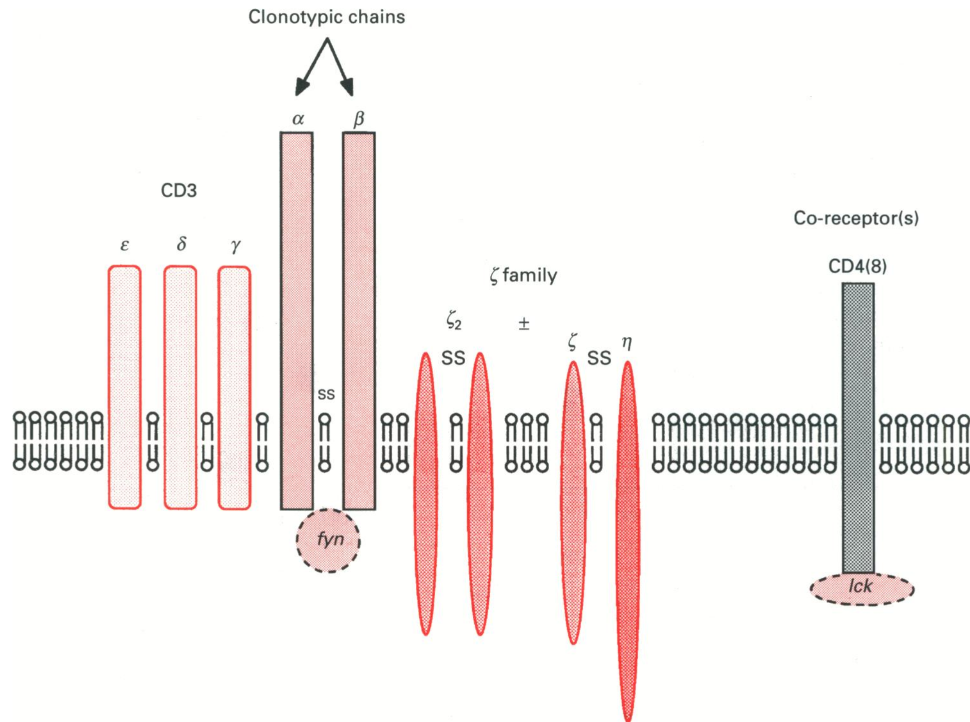

Figure 3 The T lymphocyte antigen receptor complex

Mature T cells possess antigen receptor complexes containing seven or nine components. All functional receptors possess the clonotypic α and β subunits (or γ and δ chains in the case of TcR1^{+} cells) plus the γ, δ and ϵ chains of the CD3 complex. Mature T cells can possess ζ_2 homodimers either alone or in conjunction with the $\zeta\eta$ heterodimer. The CD4 (or CD8) co-receptor is shown in association with its associated non-receptor tyrosine kinase, the pp56^{lck} product (broken circle). The pp59^{fyn} product is shown in association with the TcR/CD3 complex; the precise point of interaction with these receptor components is undefined.

1986; Ales-Martinez et al., 1991). Thus, although there is some controversy as to the extent of protein kinase C (PKC) activation in immature B cell lines, the general consensus is that the negative signals generated by ligation of the antigen receptors are likely to be due to coupling of sIg to additional (as yet unknown) transmembrane signalling pathways. The mechanisms by which the same receptor can elicit three different biological responses in a maturation state-dependent manner is one of the central problems in B cell differentiation which remains to be resolved unequivocally.

THE B LYMPHOCYTE ANTIGEN RECEPTOR

General considerations

It is intuitively obvious that mIg occupies a central position in the B cell antigen receptor, and great efforts have been made to define the structural basis for the function of mIg in this context. Analysis of the genomic organisation of the constant region of the μ heavy chain gene revealed that two exons, the M exons, contributed unique codons to the mRNA molecule directing the synthesis of the membrane form of the μ heavy chain (μ_m) (Rogers et al., 1981; Alt et al., 1981). The membrane form of the μ chain contained 25–26 hydrophobic amino acids which traversed the bilayer in a helical arrangement, and a charged cytoplasmic tail of only three amino acids, Lys-Val-Lys. The paradox presented by this information was that although this charged tail could serve to facilitate stable membrane insertion of mIg, it was evidently too small to possess any intrinsic catalytic activity. However, stimulation of B cells with anti-Ig, or in specialized antigen-specific systems with antigen itself (Grupp et al., 1987), leads to activation of the calcium-mobilizing second messenger system. Moreover, recent data indicate that tyrosine kinases, phosphatidylinositol 3-kinases (Yamanishi et al., 1992) and mitogen-activated protein kinases (MAP kinases) are also activated by perturbation of the B cell antigen receptor (Gold et al., 1992; Casillas et al., 1991). Bearing these observations in mind, several groups reasoned that the mIg molecule must be associated with other cell surface proteins to form a multi-component complex which serves to transmit signals to the B cell interior upon contact with antigen.

Lessons from the T lymphocyte antigen receptor complex

A paradigm for the existence of multi-component antigen receptor complexes involved in signal transduction in lymphocytes was provided by studies of the T cell receptor (TcR). Using mild detergent solubilization techniques followed by immunoprecipitation, it was demonstrated that the TcR contained chains concerned with antigen recognition and other non-covalently associated structures (e.g. reviewed by Frank et al., 1990). The complete TcR structure at the surface of mature T lymphocytes contains either seven or nine components (Figure 3). Mature T lymphocytes possess one of two types of clonally distributed receptor; the most abundant of these, TcR2, contains α and β chains (transmembrane glycoproteins, each of approx 45 kDa) as its clonotypic components, while the less widely expressed receptor, TcR1, possesses γ and δ chains. Whilst the clonotypic heterodimer is structurally homologous to mIg (i.e., possesses Ig superfamily domains) and is the product of genes which undergo somatic recombination and allelic exclusion, the other chains of

the TcR are invariant transmembrane proteins, composed of the CD3 ($\gamma\delta\epsilon$) complex and the ζ(ζ and η) family of proteins (Frank et al., 1990). The invariant chains of the TcR complex play roles in intracellular trafficking of the newly-synthesized receptor, and in transduction of the transmembrane signals generated by ligation of the clonotypic receptor (Frank et al., 1990; Finkel et al., 1991).

Optimal signalling via the TcR complex appears to require additional associations with appropriate co-receptors and their accessory transducing molecules. CD4 and CD8 are "co-receptors" in the antigen receptor complex. The TcR recognizes a peptide located in a peptide binding groove of a class I or II MHC antigen with low affinity and the CD4 or CD8 molecule confers stability upon the TcR–MHC complex. Non-receptor protein tyrosine kinases (PTKs) are found in association with this activated TcR–co-receptor complex. The two best characterized kinases are members of the *src* family of tyrosine kinases and are encoded by the *lck* and *fyn* cellular proto-oncogenes (Veillette et al., 1988; Samelson et al., 1990). The $pp56^{lck}$ kinase is non-covalently associated with the CD4 structure on helper T cells and the CD8 molecule on cytotoxic T cells. The second tyrosine kinase is $pp59^{fyn}$, which co-immunoprecipitates with the TcR–CD3-ζ complex (Dasgupta et al., 1992). These tyrosine kinases appear to be crucial in the coupling of the T cell antigen receptor complex to inositol phospholipid signalling events (June et al., 1990; Mustelin et al., 1990) and their activity appears to be further regulated by recruitment of the transmembrane protein tyrosine phosphatase (PTPase) CD45 to the TcR receptor complex (Koretzky et al., 1990; Alexander, 1990). That this structure plays a crucial rôle in regulation of signalling in lymphocytes is evidenced by the observation in T cells that signal transduction via the antigen receptor, but not via transfected G-protein-coupled muscarinic receptors, is abrogated in a $CD45^-$ variant of the human HPB-ALL leukaemic T cell line (Koretzky et al., 1990); similar results were observed for a plasmacytoma B cell line expressing a transfected mIg receptor (Justement et al., 1991). The transfection of a CD45-expressing plasmid into the $CD45^-$ HPB-ALL cell line corrects the defect, presumably via dephosphorylation and activation of $p56^{lck}$ (Ostergaard et al., 1989; Mustelin et al., 1989), and restores the capacity for functional signalling via the TcR. Thus, in a fashion analogous to that described for the intrinsic protein tyrosine kinase growth factor receptors, perturbation of the TcR complex leads to PTK-mediated activation of PLC (PLC-γ1) (Park et al., 1991; Secrist et al., 1991; Weiss et al., 1991), generation of $InsP_3$ and diacylglycerol, elevation of intracellular Ca^{2+} levels and activation of protein kinase C. There is, as yet, no evidence of involvement of classical heterotrimeric G proteins in linking the TcR to PLC (Graves and Cantrell, 1991; Phillips, 1991). However, recent evidence has indicated that a novel G-protein (p32) is associated with the CD4 (CD8)–$pp56^{lck}$ complex which may play a role in modulating these key early signalling events (Telfer and Rudd, 1991). Furthermore, a role for G-proteins has also been implicated by the recent finding that ligation of the TcR complex is coupled to $p21^{ras}$ activation resulting from a rapid decrease in the levels of GTPase activating protein (GAP) activity (Downward et al., 1990). The *ras* family of proteins are believed to be involved in the regulation of cellular proliferation and, thus, TcR coupling to *ras* activation would be consistent with the ability of antigen receptor-mediated signals to induce T cell proliferation.

The B lymphocyte antigen-receptor complex

Mild detergent lysis studies in the B cell system have revealed the consistent presence of two glycoproteins, found as a disulphide-bonded heterodimer, which are non-covalently associated with the mIg complex (Campbell and Cambier, 1990; Chen et al., 1990; Hombach et al., 1990; Parkhouse, 1990) (Figure 4). Both of these mIg-associated proteins have been demonstrated to be encoded by genes expressed only in B lymphocytes. The Ig-β chain, encoded by the B29 gene (Hermanson et al., 1988), has a molecular mass of 38 kDa and is a transmembrane glycoprotein. The electrophoretic properties of Ig-β are essentially identical for all isotypes studied, leading to the conclusion that mIg molecules of all classes can associate with Ig-β (Wienands et al., 1990). The Ig-α chain, also a transmembrane glycoprotein and encoded by the B cell-specific *mb*-1 gene (Sakaguchi et al., 1988), displays a range of molecular masses when analysed by two-dimensional electrophoresis. This observation initially attracted great interest as it suggested the possibility that multiple forms of the Ig-α chain existed which associated with heavy chains in an isotype-specific manner. This notion has been shown to be incorrect by transfection of "tagged" genes which demonstrate that the differences in electrophoretic mobility of Ig-α molecules are entirely attributable to variability in N-glycosylation patterns, and that there is only one Ig-α form which can associate with all mIg isotypes (Venkitaraman et al., 1991). The stoichiometry of the complex formed between the clonotypic chains of the receptor and the Ig-α–Ig-β heterodimer remains to be defined.

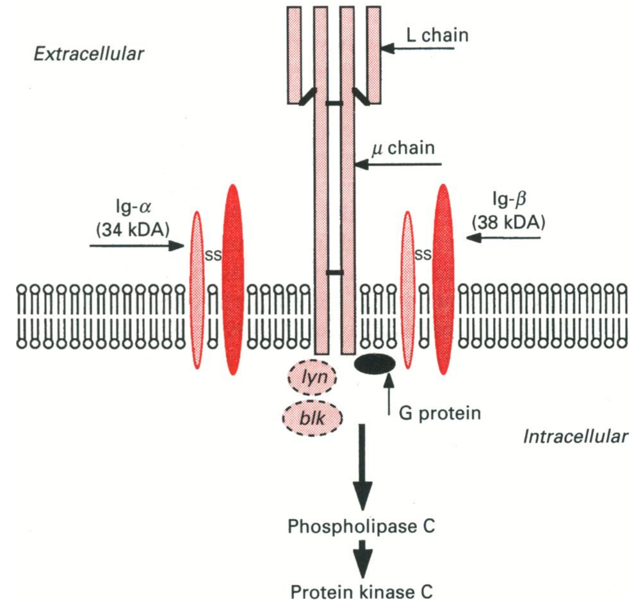

Figure 4 The B lymphocyte antigen receptor complex

The B cell antigen receptor complex is shown for a mIgM molecule; a similar layout is likely to apply to other Ig isotypes. The clonotypic chains of the receptor are provided by the μ heavy chain and light chain molecules, and the Ig-α and Ig-β associated proteins are shown as shaded ellipses. The associations with the *src*-like kinases, *blk* and *lyn*, and with a G protein are also shown. No definitive stoichiometric data are available for numbers of $\alpha\beta$ heterodimers per clonotypic unit.

Considerable excitement surrounds the possible functions of Ig-α and Ig-β. The two most likely functions of these mIg-associated glycoproteins are in signal transduction and in assembly and transport of the B cell antigen-receptor complex. Early evidence for a role for Ig-α in transport was provided by transfection studies in the J_{558} plasmacytoma (these B cells do not synthesize heavy chains, but do produce λ_1 light chains necessary for assembly of complete Ig molecules). Introduction of the secretory form (μ_s) of the μ heavy chain resulted in efficient

secretion of IgM (Hombach et al., 1988). However, transfection of μ_m plasmids resulted in synthesis of the μ_m heavy chain, but no appearence of mIgM at the surface of the cells: surface expression could only be achieved if *mb*-1 (i.e. Ig-α) was also transfected into the J_{558} cells together with the μ_m constructs (Hombach et al., 1990). This result led to the proposal that Ig-α was mandatory for surface expression of all Ig isotypes. While this maxim is true for IgM, it is not an universally applicable rule, since detailed transfection experiments have demonstrated that IgD (Wienands et al., 1990; Venkitaraman et al., 1991) and certain IgG subclasses can be efficiently expressed at the cell surface in the absence of the Ig-α protein (Venkitaraman et al., 1991).

Both Ig-α and Ig-β are transmembrane glycoproteins with sizeable intracellular domains. However, inspection of the sequences of the cytoplasmic domains reveals an absence of any motifs which suggest that either Ig-α or Ig-β possesses intrinsic catalytic activity. Indeed, the available data suggest no definitive role for Ig-α and Ig-β as direct couplers of mIg to the B cell signal transduction machinery. The cytoplasmic sequences of Ig-α and Ig-β do, however, possess motifs which are also found in the cytoplasmic domains of certain of the CD3 complex components. Moreover, there is abundant evidence that both Ig-α and Ig-β are substrates for protein kinases, including protein tyrosine kinases (Campbell and Cambier 1991; Gold et al., 1991). The involvement of PTKs and PTPases in the activation of T lymphocytes is well established, and studies in B cells have also strongly implicated activation of PTKs, both in intact cell and isolated membrane systems (Campbell and Sefton, 1990; Gold et al., 1990). Thus, *src*-like kinases such as *fyn* , and others which are apparently unique to B cells, the *blk* and *lyn* kinases, have recently been shown to be associated with the mIg complex and coupled to mIg activation (Yamanishi et al., 1991; Burkhardt et al., 1991; Gold et al., 1991). One or more of these PTKs is likely to be responsible for tyrosine phosphorylation of the mIg-associated molecules following receptor crosslinking, and it is possible that CD45 (Justement et al., 1991) is involved in the dephosphorylation, and hence modulation, of cellular signalling via Ig-α and Ig-β.

SIGNAL TRANSDUCTION VIA THE B LYMPHOCYTE ANTIGEN RECEPTOR

In a normal individual, each B cell possesses an unique receptor for antigen and, even in an on-going response to deliberate immunization, the frequency of B cells specific for a given antigen is of the order of 10^{-7}. Except in specialized normal B cell systems (Snow et al., 1983; Pike and Nossal, 1985; Grupp et al., 1987), or in B cell lymphomas such as the CH12 series whose antigen receptor has a defined specificity (Mercolino et al., 1986), the use of antigen to stimulate the B cell antigen receptor is quite unrealistic. Consequently, the majority of studies have been performed using anti-Ig reagents to "mimic" the effect of antigen. As will be noted below, this approach is not without inherent complications of its own, but consistent patterns of data have emerged in both human and murine models and these are detailed below.

G proteins and tyrosine kinases

The antigen receptors (mIgM and mIgD) on B cells are coupled to the activation of at least two early transmembrane signalling events: (i) the PLC-mediated hydrolysis of PtdInsP_2 to generate the intracellular second messengers InsP_3 and diacylglycerol (Bijsterbosch et al., 1986) and (ii) activation of PTK activity leading to the tyrosyl-phosphorylation of target proteins (Campbell and Sefton, 1990). The identity of the PTK(s) involved has not yet been defined, but is likely to be one or more of the *src*-related PTKs, *blk*, *lyn* and *fyn* recently shown to be associated with the mIg receptors (Yamanishi et al., 1991; Burkhardt et al., 1991). Another early event in B cell activation is the redistribution of PKC to the plasma membrane where up to nine proteins have been identifed as substrates, including class I, but not class II, MHC antigens (Burke et al., 1989).

There is, at present, considerable debate concerning the coupling of the antigen receptors to PLC activation in B cells. The observation that the kinetics of PLC activation following crosslinking of the antigen receptors are typical of classical G-protein-coupled calcium-mobilizing receptors and experiments demonstrating disruption and functional GTP-dependent reconstitution of the mIg-signalling pathway in permeabilized cells support the hypothesis that both classes of antigen receptors are regulated by a toxin-insensitive G protein (Gold et al., 1987, Harnett and Klaus, 1988), which may belong to the Gq subclass of toxin-insensitive G-proteins recently shown to be involved in regulation of PLC activity (Smrcka et al., 1991; Taylor et al., 1991). However, recent data detailing the structural properties of the G-protein-coupled receptor superfamily, notably the pre-eminence of a seven membrane-spanning loop structure, sparked off a controversy concerning the role of classical heterotrimeric G-proteins in the coupling of the lymphoid antigen receptors to PLC. Thus, despite evidence that other classes of receptors with single transmembrane-spanning regions, such as the TNFα receptor, can be G-protein regulated (Yanaga et al., 1992), together with the possibility that the antigen receptor-associated accessory molecules would provide candidates for coupling elements, many workers began to consider it unlikely that the antigen receptors, with their pairs of single transmembrane regions and short cytoplasmic tails, would be able to couple to a G-protein. The debate was further fuelled by the consistent failure to demonstrate classical G-protein coupling of the TcR (Graves and Cantrell, 1991; Phillips et al., 1991), which led to the acceptance of the proposal that the TcR is coupled to PLC activation by PTK activity in a manner similar to that described for receptors such as those for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) which possess intrinsic tyrosine kinase activities.

The discovery of sIg-mediated PTK activation (Gold et al., 1990; Campbell and Sefton, 1990) and studies using PTK inhibitors to investigate mIg/PLC coupling (Carter et al., 1991; Lane et al., 1991), suggested that similar events could also occur in some B cell lines. Studies with PTK inhibitors, such as genistein, herbimycin and tyrphostin, showed that these reagents could inhibit anti-Ig-mediated InsP_3 generation and calcium mobilization in human B cells and lymphoblastoid cell lines such as the Daudi cell line [generally following a long-term pre-incubation (16–20h) of cells with the inhibitor]. In addition, Carter et al. (1991) demonstrated that ligation of mIg could lead to phosphorylation of PLC-γ1 in a human B lymphoblastoid cell line. These findings, although interesting, should be assessed carefully, as Hempel and DeFranco (1991) have shown that whilst the predominant forms of PLC expressed in a range of murine B cell lines are the α and γ2 isoforms, PLC-γ1 levels of expression are generally very low or, indeed, absent in some lines. In addition, the kinetics of Ca^{2+} mobilization by PTK-coupled receptors are typically slower than those observed when signals are transduced via classical G-protein-coupled calcium-mobilizing receptors (Margolis et al., 1990), and the rapid kinetics of mIg-activated normal B cells would therefore be more consistent with coupling via a classical G protein system rather

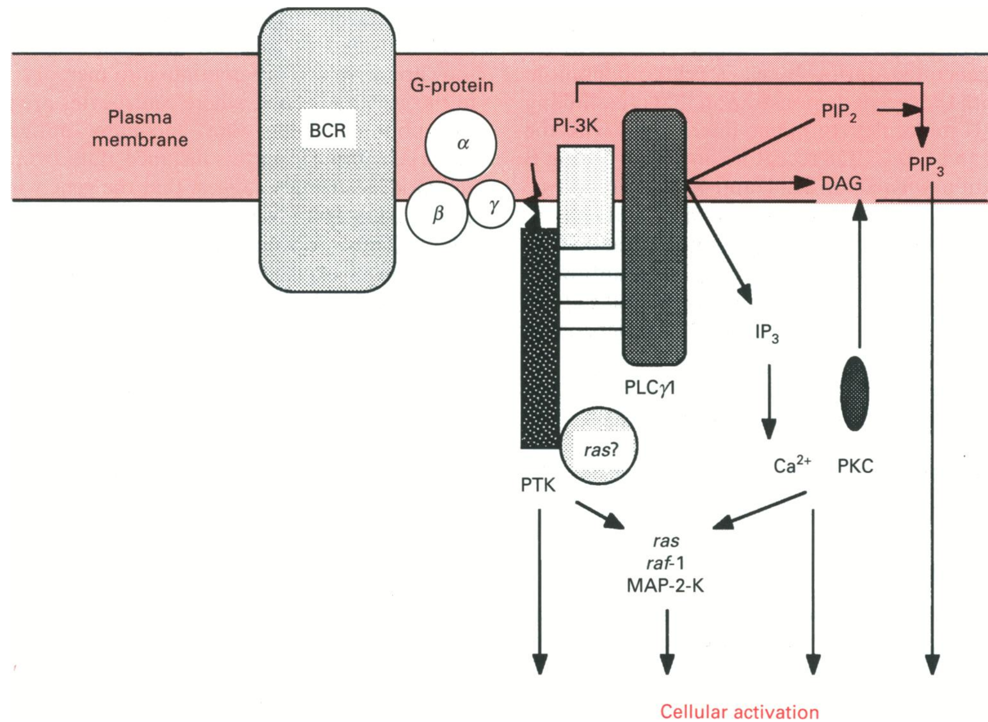

Figure 5 Signalling pathways linked to the B cell antigen receptor

"BCR" in this Figure represents the clonotypic and associated structures depicted in Figure 4. The spatial linkage between the associated heterotrimeric G protein and the effector systems [PTK, phosphatidylinositol 3-kinase (PI-3K) and PLCγ1] are detailed, together with their relationship to likely downstream effectors necessary for cellular activation. Abbreviations: PIP_2, phosphatidylinositol bisphosphate; PIP_3, phosphatidylinositol trisphosphate; DAG, diacylglycerol; IP_3, inositol trisphosphate.

than via a PTK system. Furthermore, under the conditions necessary to induce inhibition of mIg-mediated InsP_3 production by PTK inhibitors in normal murine B cells, these reagents also induce toxic and/or nonspecific effects on other cellular kinases such as PKC and the phosphatidylinositol kinases (M. M. Harnett, unpublished work). Indeed, recent data demonstrate that those effects of the PTK inhibitors which have been interpreted in the above studies in terms of the disruption of receptor–PLC coupling could simply reflect the ability of these reagents to block production of PtdInsP_2, the PLC substrate (M. M. Harnett, unpublished work). Thus, mIg-mediated PTK activation may play an important role(s) in B cell activation, although it does not appear to be the sole or even major regulatory element underlying coupling of the antigen receptors to PLC in normal resting B cells. However, in view of the increasing evidence supporting G-protein regulation of intrinsic PTK growth factors in primary cells, rather than the PTK coupling observed in transformed cells or in cell lines that overexpress receptors (Liang and Garrison, 1991; Yang et al, 1991), it is possible that the antigen receptors are differentially coupled to various PLC isoforms via G-proteins and/or PTKs depending on their state of activation or differentiation. Finally, PTKs and G-proteins need not play mutually exclusive roles in the regulation of mIg/PLC coupling (Figure 5). Indeed, recent studies by Roifman and co-workers may finally reconcile the conflicting evidence supporting roles for both G-proteins and PTKs in mIg/PLC coupling. These authors now have evidence that in human peripheral blood B lymphocytes, mIg is coupled to PTK activation, tyrosine phosphorylation of PLC-γ1 and inositol phosphate generation via a pertussis toxin-sensitive G-protein (Melamed et al., 1992) in a manner reminiscent of the involvement of Gi in EGF-mediated activation of PLC-γ1 in primary rat hepatocytes (Yang et al., 1991).

Models of T cell-dependent B cell activation

Anti-Ig antibodies are most likely to provide a model for polyclonal B cell activation by type-2 T cell-independent (TI-2) antigens, which are typically large polymers, with repeating epitopes, capable of efficient crosslinking of sIg receptors. It is still a matter of debate how these effects of anti-Ig relate to those of soluble T cell-dependent antigens which are likely to be rather poor crosslinking agents and which do not cause substantial B cell activation in the absence of T cells. However, evidence from studies using hapten–protein conjugates suggests that that T cell-dependent antigens may produce a limited inositol phospholipid turnover leading to abortive activation of B cells. This abortive entry into the cell cycle may be important since the resultant highly-efficient internalization of antigen followed by proteolysis and re-expression of antigen-derived peptides in association with MHC Class II molecules (Germain and Hendrix, 1991) is likely to promote B cell–T cell co-operation; that is, abortive activation promotes the conditions necessary for "cognate" recognition of antigen (Noelle and Snow, 1991). Secondary intercellular interactions between adhesion and accessory molecules (see below) may then generate 'bi-directional' signalling in which the T helper cell is stimulated to proliferate and release cytokines which, in turn, act to amplify the B cell response. The finding that the T cell-derived cytokine IL-4 could act, *in vitro*, as a co-mitogen with submitogenic concentrations of anti-Ig (Howard et al., 1982) (which induce a low level of InsP_3 and diacylglycerol generation) led to the current hypothesis that IL-4 (and possibly

other lymphokines generated by antigen-presenting cells?) interacts with the mIg signalling cascade to "prime" the B cell for additional signals generated during B cell–T cell co-operation. This is consistent with the recent demonstration that crosslinking of the MHC Class II molecules (to mimic interactions with the TcR–CD3 complex and CD4/CD8 receptors on T cells during B cell–T cell co-operation *in vitro*) on IL-4/anti-Ig-primed murine cells, drives all resting B cells into S phase (Cambier and Lehman, 1989). Provision of additional lymphokines (e.g. IL-4 and IL-5) at this stage drives the differentiation of B cells into antibody-secreting plasma cells (Cambier and Lehman, 1989). This murine experimental model of B cell activation by T cell-dependent antigens has been strongly supported by reconstitution experiments which show that incubation of B cells with fixed, pre-activated T cells (or even membranes from pre-activated T cells) in the presence of IL-4 and IL-5 is sufficient to induce B cell proliferation and differentiation into antibody-secreting cells (Brian, 1988; Hodgkin et al., 1990; Noelle et al., 1991).

Patterns of gene expression

Stimulation of B cells with antigen or anti-Ig causes several changes in the pattern of gene expression in the cell. The most pronounced of these is the massive up-regulation of class II MHC antigens which, as noted above, is important in facilitating T cell–B cell co-operation. Other rapid changes include increases in levels of c-*fos*, *Egr*-1 and *c-myc* mRNA (Cambier and Campbell, 1990), all of which appear to be linked to the calcium-mobilizing second messenger system. Two inducible genes are particularly noteworthy in terms of the nature of the B cell response to stimulation of the antigen receptor.

Immature B cells, which are $mIgM^+/mIgD^-$, respond negatively to stimulation of the antigen receptor (i.e. they become anergic or enter apoptosis), while the next differentiation stage, the mature B cell ($mIgM^+$ / $mIgD^{++}$) becomes activated by perturbation of the antigen receptor. A possible molecular explanation for this phenomenon, in the murine model, lies in the capacity of mature B lymphocytes to express the *Egr*-1 (*e*arly growth *r*esponse) gene in response to anti-Ig stimulation. The *Egr*-1 gene product is a transcriptional regulatory factor, which is induced by anti-Ig (or phorbol ester) stimulation in mature but not immature B cells; it is also absent from B cell precursor cells in the bone marrow, and cannot be induced in IgM^+/IgD^- B cell lymphomas such as the WEHI-231 line. The inability to express *Egr*-1 is associated with a lack of proliferation in response to antigen receptor stimulation (Seyfert et al., 1991). The mechanism by which immature B cells fail to respond to anti-Ig stimulation at the level of expression of *Egr*-1 does not involve the presence or absence of differentiation-stage-specific *trans*-acting factors (as is the case for control of MHC class II expression by IL-4 in murine B cells; see section below), but is dependent upon the state of methylation of the promoter region of the *Egr*-1 gene itself (Seyfert et al., 1991). Thus, in immature B lymphocytes, the *Egr*-1 promoter is heavily methylated, while B cell lines representative of the mature B cell phenotype (e.g. BAL-17) and normal splenic B lymphocytes possess a hypomethylated *Egr*-1 promoter region. Interestingly, treatment of the immature B lymphoma line WEHI-231 with 5-azacytidine, a non-specific inhibitor of methylation, leads to hypomethylation of the *Egr*-1 promoter region in these cells which results in the cells synthesizing *Egr*-1 mRNA upon anti-Ig stimulation. Thus, the methylation status of the *Egr*-1 gene is a reflection of the state of maturity of early antigen-dependent B cells, and this gene must be in a hypomethylated state before B cells can respond to anti-Ig stimulation by cellular activation.

Once mature $mIgM^+$ / $mIgD^{++}$ mature B lymphocytes have been activated by antigen, they face a second selection step if they are driven to differentiate into memory B cells. This occurs in the germinal centre where centrocytes are programmed to die by apoptosis unless they encounter antigen (and other appropriate stimuli). In this instance, data from human tonsillar B cell models strongly suggest that the gene which is necessary for escape from apoptosis is the *bcl*-2 oncogene (Liu et al., 1991a,b, 1992). Thus, stimulation of centrocytes with anti-Ig causes induction of expression of *bcl*-2 within 4 h (Liu et al., 1991b): other signals which can promote *bcl*-2 expression in centrocytes include the combination of sCD23 and IL-1α (Liu et al., 1991a) and ligation of the CD40 antigen on the centrocyte surface (Liu et al., 1991b). Thus, the stimuli which induce *bcl*-2 expression in centrocytes therefore correlate exactly with those which rescue the same cells from apoptosis. Interestingly, certain lymphomas of centrocytic cells display a chromosomal translocation which places a section of chromosome 18 containing the *bcl*-2 oncogene under the influence of the *Igh* enhancer on chromosome 14 (Tsujimoto et al., 1985). Centrocytic cells bearing this translocation are neoplastic and survive without the need for signals from antigen receptors or FDCs by virtue of their constitutive expression of the *bcl*-2 protein. Finally, transfection of *bcl*-2 into *bcl*-2^- B cell lymphomas renders such cells refractory to apoptosis as a consequence of serum starvation (Henderson et al., 1991).

CYTOKINES AND B LYMPHOCYTE SIGNALLING

Introduction

All stages of B lymphocyte activation, growth and differentiation are regulated by cytokines. Thus, in the bone marrow, the antigen-independent phase of B cell differentiation is heavily dependent upon cytokines derived from stromal cells (e.g. IL-7 and IL-11) and, to a lesser extent, by cytokines derived from T cells (reviewed by Callard, 1990). Once a functional antigen receptor is expressed upon the B cell surface and the cell is capable of responding to antigen, a range of cytokines tightly regulates the precise immunological characteristics of the behaviour of the B cell clones recruited to the response. The majority of the cytokines which are important in the antigen-dependent phase of B cell differentiation are derived from helper T lymphocytes: for example, whilst IL-2 is involved in regulating growth of cycling B cells (Jelinek et al., 1986) and accelerating antibody secretion [as is IL-6 (Hirano et al., 1986)], other cytokines such as IL-4 and IFNγ direct molecular processes in the B cell, for example, by positively influencing heavy chain isotype switching (Lutzker et al., 1988; Rothman et al., 1988). Many B cell responses to cytokines are not a reflection of stimulation with one cytokine alone, but are indicative of the combined effects of several cytokines. Thus, certain cytokines can interact synergistically upon B cells, while others, such as IL-4 and IFNγ (Rabin et al., 1987) or IL-4 and IL-2 (Jelinek and Lipsky, 1987; Lorente et al., 1990), appear to be mutually inhibitory. All of these phenomena pose interesting questions at the level of the nature of the cellular signals generated upon interaction of cytokines with their complementary cell surface receptors, and set further challenges for an understanding of how such cellular signals might interact, positively or negatively, within the B lymphocyte to elicit the appropriate biological response.

Recent advances in cloning of the receptors for a number of cytokines has improved our knowledge of the structural biochemistry of the receptors, but has not yet yielded definitive data to explain their interaction with cellular signalling systems. The data suggest that cytokine receptors may be conveniently grouped

into four main categories. The first are members of the Ig-like superfamily, and are exemplified by the IL-1 receptors which are discussed below. A second group are classical G-protein coupled receptors. An example of such a cytokine receptor is that for the 9 kDa cytokine IL-8 (Oppenheim et al., 1991), which is present as a single class of high-affinity receptors (Samanta et al., 1989) linked to phospholipase C via a pertussis toxin-sensitive G-protein, with ligation of the receptor triggering Ca^{2+} mobilization and activation of PKC (Dewald et al., 1988). The cloning of the IL-8 receptor from a cDNA library of neutrophil-specific genes (Holmes et al., 1991; Murphy and Tiffany, 1991; Thomas et al., 1991) reveals it to be a seven-transmembrane-domain type, prototypic G-protein-coupled receptor. The third group of receptors expressed on B cells are those which possess intrinsic tyrosine kinase catalytic domains in their intracellular domains, as opposed to the antigen receptors and some cytokine receptors which appear to be coupled to non-receptor PTK activities. Thus, activated murine T and B cells have been shown to mount a mitogenic response to insulin stimulation (Snow et al., 1980), and functional receptors for stem cell factor are present on some B lymphocyte types. The final group of cytokine receptors, to which many interleukin receptors belong, is the haematopoietin receptor superfamily (HRS) (Bazan, 1989). While the signal transduction pathways for a few systems have been rigorously characterized (e.g. for the IL-8 receptor), a consistent pattern has not emerged for signal transduction within or amongst the cytokine receptor families. Heated debate regarding lineage-specific (IL-1) and species-specific (IL-4) cellular signalling mechanisms surrounds certain individual cytokine/receptor systems.

Receptors of the Ig-like superfamily: IL-1

Members of the Ig-like superfamily include the receptor(s) for IL-1. The key feature of these receptors is that they contain structures characteristic of the prototypic Ig domain. Thus, members of this family have one or more protein domains which are approximately 110 amino acids in size and display good primary structural similarity to immunoglobulin molecules, including a single disulphide bond enclosing 65–75 residues. At the higher structural levels, such domains possess seven anti-parallel β strands as their principal secondary structural motif, and have the β strands arranged in two faces of four and three strands linked by a single disulphide bond (Williams and Barclay, 1988). This structural motif is widely represented in the cell surface molecules of the immune and neural systems.

IL-1 is a highly pleiotropic cytokine, influencing the growth and differentiation of a wide range of cell types (reviewed by Dinarello et al., 1989). The cytokine is found in two forms, IL-1α (159 amino acids) and IL-1β (153 amino acids), both of which are initially found as cell-surface-associated molecules before being cleaved to generate soluble cytokine (Martin and Resch, 1988). Both IL-1α and IL-1β display activity towards a similar range of target cells. Both the T cell, type I (Sims et al., 1989), and the B cell, type II (McMahan et al., 1991), IL-1 receptors have now been cloned. The IL-1 receptor found on human T lymphocytes and fibroblasts comprises 552 amino acids (557 in the mouse), and the layouts of the domains in both species are similar (Sims et al., 1989). Thus, there is an extracellular binding region composed of three Ig-like domains, a single transmembrane sequence, and a large (215 residues) cytoplasmic tail, with some features conserved in many nucleotide binding proteins; there are no motifs consistent with the IL-1 receptor cytoplasmic domain possessing intrinsic protein kinase activities (Sims et al., 1989). The B cell type receptor has a similar extracellular region, with three Ig-like domains, also possesses a single transmembrane region, but has a small 29-amino-acid cytoplasmic tail region (McMahan et al., 1991). Both types of receptor can bind both IL-1α and IL-1β. Interestingly, type I receptors show a single class of binding sites for IL-1α, but two sites for IL-1β, while the type II receptor displays the reverse characteristics (McMahan et al., 1991). Both receptors are linked to pertussis toxin-sensitive G proteins (Mizel, 1990; O'Neill et al., 1990) and, whilst they activate the Na^+/H^+ antiporter, neither receptor mediates an increase in intracellular Ca^{2+} levels. Finally, both receptors undergo receptor-mediated endocytosis following ligand binding, and there is strong evidence to suggest that the receptor–ligand complex may ultimately translocate to the nucleus via an endosomal compartment (Grenfell et al., 1989; Curtis et al., 1990).

Lineage-specific IL-1-triggered signalling pathways

Controversy surrounds the precise downstream signal transduction mechanisms activated in IL-1-sensitive cells (Mizel, 1990; O'Neill et al., 1990). The IL-1-driven transcriptional activation in 70Z/3 pre-B cells is mediated by the NF-κB *trans*-acting factor, and can be mimicked by exposure of the cells to forskolin via protein kinase A activation. Together with studies indicating that IL-1 receptor activation leads to elevations in cyclic AMP levels in other lymphocyte models, the data from the 70Z/3 pre-B cell model suggest that IL-1 mediates its effect upon NF-κB activity via elevation of cyclic AMP and subsequent activation of protein kinase A (Mizel, 1990). This suggestion is not, however, supported by data from other studies of the mechanism of action of IL-1 upon fibroblasts (O'Neill et al., 1990). Thus, fibroblasts respond to IL-1 stimulation by phosphorylation, upon serine residues, of the 80 kDa type I IL-1 receptor itself and the EGF receptor. This effect cannot be mimicked by forskolin, suggesting a lack of involvement of protein kinase A, and also fails to be induced by exposing the fibroblasts to phorbol esters. The hypothesis that PKC is not involved in the signal transduction pathways activated by IL-1 in fibroblasts is further supported by the observation that chronic treatment with phorbol ester, which down-regulates protein kinase C activity (O'Neill et al., 1990), fails to inhibit the ability of IL-1 to stimulate phosphorylation of the IL-1 and EGF receptors. This suggests the involvement of a distinct kinase(s) linked to the IL-1 receptor, and precedents for this are found in the IL-2 receptor system discussed below.

The data from analysis of IL-1 receptor-mediated signal transduction in pre-B cells and fibroblasts raises the possibility that distinct cell lineages possess structurally-different IL-1 receptors which are, in turn, linked to non-identical signal transduction pathways. Further circumstantial support for such a notion is provided by the observation that in certain IL-1-sensitive cell types, diacylglycerols are generated from different lipid sources (O'Neill et al., 1990). Thus, phosphatidylcholine appears to be the principal source of diacylglycerol in T cells, phosphatidylinositol serves the equivalent function in macrophages, while in mesangial cells phosphatidylethanolamine is the predominant source of ligand-sensitive diacylglycerol.

Cytokine receptors of the haematopoietin receptor superfamily

The final group of cytokine receptors is the haematopoietin receptor superfamily (HRS). This superfamily was initially described by Bazan (1989), D'Andrea et al. (1989) and Cosman et al. (1989). The HRS is divisible into two subgroups which, while having many features in common, also possess characteristic structural differences. Type I receptors (which for con-

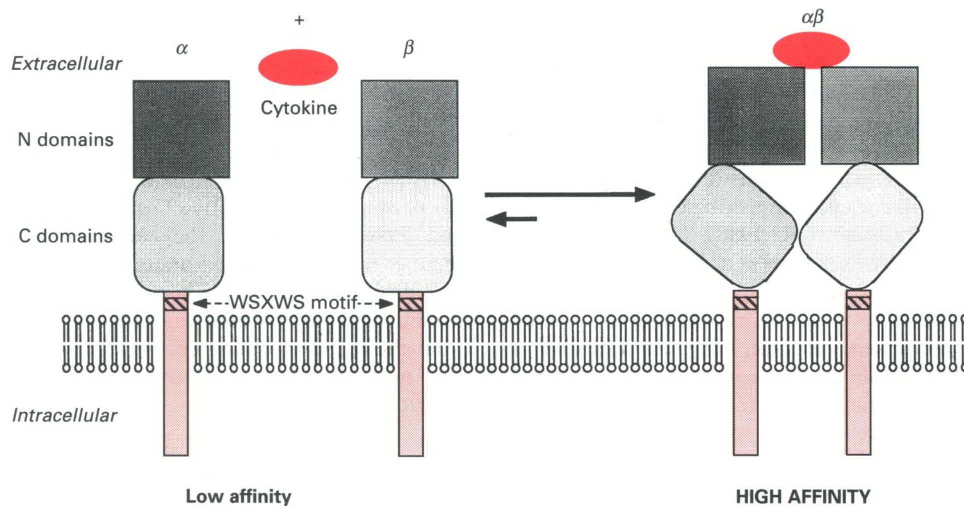

Figure 6 A prototypic HRS cytokine receptor

A prototypic HRS receptor is shown. The N domains are shown as squares, the C domains as oblongs, and the cytoplasmic tails as open rectangles; the WSXWS motif is shown as a hatched box. Based on the IL-3 or IL-6 receptor models (see the text for details), the cytokine (filled ellipse) binds to the α chain of the receptor driving association with the β subunit. The association is via the C domains. After cytokine binding the equilibrium of the reaction lies to the right, and signalling proceeds principally via the high-affinity receptor complex.

venience we shall refer to as "HRS" receptors) include receptors, or one or more components of receptors, for IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF, erythropoietin, prolactin and growth hormone (GH). Type II receptors includes receptors for the interferons and for tissue factor.

In general terms, all HRS receptors are transmembrane glycoproteins, possessing two extracellular domains, a short transmembrane region and a large cytoplasmic tail. The recent crystallization and determination of the structure of a complex of GH and its receptor at a resolution of 2.8 Å (De Vos et al., 1992) has suggested many interesting properties for the HRS receptors and their ligands in terms of conserved primary and higher structural features. For example, the extracellular surface of the receptor comprises two domains, N and C (Bazan, 1989), which are composed of anti-parallel β strands arranged in faces of three and four strands. The N domain of the GH receptor possesses three disulphide bonds, and the placement of four of the cysteines participating in formation of these disulphide bonds is highly conserved in all HRS receptors; no disulphide bond is found in the C domain. The HRS receptors also possess structural characteristics akin to type III fibronectin domains, which could suggest a adhesion function of these molecules or may simply reflect the evolutionary origins of members of this receptor group (Bazan et al., 1990). The final characteristic feature of note in the extracellular surface of the HRS receptors is the finding of a very highly conserved pentapeptide motif (Trp-Ser-Xaa-Trp-Ser, the WSXWS motif) located close to the transmembrane region in the primary structure. The intracellular domain is of variable size and shows little conservation within the HRS. In terms of activation of cellular signalling pathways, there are few notable structural features in the intracellular domains of HRS receptors, and these display no motifs which indicate that they possess catalytic domains. There are, however, several good candidate residues which could be substrates for protein kinases, and there is evidence that ligation of several of the cytokine receptors of this family leads to p21^{ras} activation (Satoh et al., 1991).

Perhaps the most important finding from the GH model is that the stoichiometry of the complex is one ligand molecule to two receptor molecules. The data also indicate that ligand binding is accomplished by the N domains, and the binding causes a very close association between C domains to take place (Figure 6). Moreover, binding appears to show a degree of co-operativity, with binding to one receptor site being necessary for binding to a second site (Cunningham et al., 1991). In addition, occupation of the two receptor chains seems to confer stability on the receptor–ligand complex which may have important consequences for initiation of signal transduction via the dimerized receptors: this is consistent with observations on the EGF, insulin and PDGF receptors, where maximal activation of their intrinsic tyrosine kinase activities depends on receptor dimerization. The following examples of cytokine receptors functioning in B cells serve to illustrate this point. However, unlike the growth factor and GH systems, the cytokine receptor systems currently best understood show receptor heterodimerization.

IL-2: receptor heterodimerization

The IL-2 receptor is a true cytokine receptor complex, possessing two defined ligand binding subunits, and a range of associated proteins (Figure 7). The two ligand-binding chains are both transmembrane glycoproteins, and both make intimate contact with the ligand itself (Teshigawara et al., 1987; Robb et al., 1987; Smith, 1989). The best characterized of the two subunits is a 55 kDa glycoprotein originally defined on activated T cells (variously called Tac antigen, CD25 or IL-2Rα), which is not an HRS receptor and which is inducible by a variety of stimuli on T lymphocytes including perturbation of the antigen receptor and the cytokines IL-1, IL-2, IL-6 and TNFα (reviewed by Greene et al., 1989; Smith, 1989; Ullman et al., 1990). IL-2Rα is also inducible in B cells, but with an apparently distinct range of cytokines. Thus, IL-4 appears to be the crucial cytokine in human tonsillar B lymphocytes (Butcher et al., 1990; Butcher and Cushley, 1991; Tomizawa et al., 1991; Zola et al., 1991), while IL-5 occupies a central role in murine B cells (Loughnan and Nossal, 1989). The second component is the 75 kDa IL-2Rβ subunit (Tsudo et al., 1986; Takeshita et al., 1989) which is

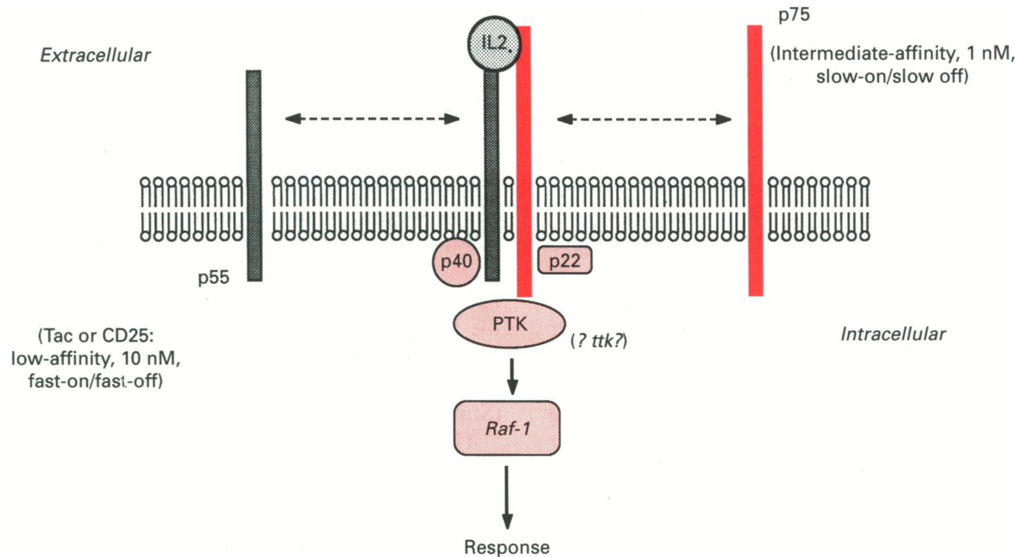

Figure 7 The IL-2 receptor complex

The properties of the two ligand-binding chains, p55 (α) and p75 (β), are indicated at either end of the Figure. In the presence of ligand, both chains bind IL-2 (see Figure 6 for the general case) and signals are transduced. The complex of α and β has a fast-on/slow- off character with high affinity (10 pM). The relationship of the high-affinity receptor to defined associated chains and enzymes involved in the signalling cascade [i.e. protein kinases (possibly the novel *ttk* activity) and c-*raf*-1] are also indicated.

constitutively expressed on lymphocytes, and is an HRS receptor (Hatakayama et al., 1989). A range of studies have illustrated a number of other proteins associated with the IL-2 receptor. One of these membrane proteins has been unequivocally identified as intracellular adhesion molecule-1 (ICAM-1, CD54) in both human and murine systems (Burton et al., 1990; Sharon et al., 1990). Radioligand cross-linking experiments have identifed the existence of two other proteins of 22 kDa and 40 kDa in murine B cells which are of unknown function (Saragovi and Malek, 1990). Scatchard analyses of IL-2 receptors on T and B cells reveal three distinct families of IL-2 binding subunits which can be explained in terms of the binding characteristics of IL-2 for each of the two receptor subunits (Teshigawara et al., 1987; Smith, 1989; Greene et al., 1989). Thus, IL-2Rα binds IL-2 with low affinity and IL-2Rβ with intermediate affinity, but the non-covalent complex of the two chains gives rise to the high-affinity receptor complex which is most likely to bind IL-2 at physiological concentrations. By analogy with the GH receptor system, IL-2Rα would bind ligand first, making the primary interaction necessary for binding of a second site on the ligand to IL-2Rβ. In this instance, distinct sites on the IL-2 molecule would be expected to make contact with unique motifs on the α and β subunits. Finally, there are recent data from human T cell lines which report the involvement of a 65 kDa component of the IL-2 receptor complex which is necessary for maximal ligand binding and activation of signal transduction (Arima et al., 1992).

At present, no consistent pattern has emerged for activation of cellular signalling pathways by IL-2 in lymphocytes (Mills et al., 1991). For example, although it is generally accepted that activation of the IL-2R does not cause the hydrolysis of PtdInsP_2leading to the generation of the intracellular second messengers, Ins$P_3$and diacylglycerol, IL-2 has been reported to induce the membrane translocation, and hence activation, of PKC (Saltzmann et al., 1989: Merida and Gaulton, 1990; Farrar and Anderson, 1985). It has also been reported that IL-2 receptor activation may lead to induction of signal transduction pathways involving cyclic AMP (Wickremasinghe et al., 1987) and glyco-phosphatidylinositol (GPI) (Eardley and Koshland, 1991). The loss of GPI molecules, presumably by action of a PLC, was paralleled by an increase in intracellular levels of inositol-phosphoglycan: diacylglycerol, in the form of myristoyl diacylglycerol, also accumulated, but was converted to myristoyl phosphatidic acid. GPI lipid release appears to be physiologically relevant to IL-2-driven T lymphocyte proliferation, since (i) IL-2 has been shown to promote loss of GPI molecules within 30 s of ligand binding at concentrations where only the high-affinity IL-2 receptor would be predicted to be occupied (Merida et al., 1990) and (ii) addition of one of the breakdown products, inositolphosphoglycan, to T cells synergized with IL-2 in promotion of cellular growth without altering the levels of IL-2 receptors expressed (Merida et al., 1990).

There is now good evidence to show that, in T cells, the IL-2 receptor is coupled to tyrosine kinases including $pp56^{lck}$ via the IL-2Rβ subunit (Hatekayama et al., 1991) and possibly $pp59^{fyn}$ (Mills et al., 1991). Recent data, in a *lck*-negative pre-B cell line, have demonstrated that the IL-2Rβ subunit can activate the B cell-specific $pp53/56^{lyn}$ kinase (Torigoe et al., 1992), suggesting lineage specificity in activitation of *src* family non-receptor associated PTKs by IL-2. In T cells, the IL-2 receptor also seems to be associated with a member of the recently described *ttk* family (*t*yrosine-*t*hreonine *k*inase), although it remains unclear if this is a direct coupling, or if another system links the receptor structure to the *ttk* activity (Mills et al., 1991). IL-2 binding is also linked to increases in the activity of phosphatidylinositol bisphosphate 3-kinase (PI-3-kinase) within 1 min of ligand binding in human peripheral blood T lymphoblasts (Remillard et al., 1991), and to activation of $p21^{ras}$, possibly either via direct tyrosine phosphorylation of $p120^{GAP}$ or by phosphorylation of intermediary regulatory proteins such as p62 and p190 (Downward et al., 1992). IL-2 stimulation of T cells also leads to tyrosine phosphorylation of the product of the c-*Raf*-1 oncogene, a 72–74 kDa protein which possesses serine-threonine protein kinase activity (Turner et al., 1991). The precise sequence and interaction of the above tyrosine kinase-mediated signalling

events is, as yet, not clear. For example, it is conceivable that c-*raf* acts downstream of p21^{ras} since in fibroblasts, p21^{ras} has been shown to modulate the function of c-*raf* (Downward et al., 1992). The central role of the tyrosine kinases in IL-2-driven cellular signalling events is underscored by the finding that exposure of cells to tyrphostins or genistein prior to IL-2 stimulation abrogates phosphorylation of IL-2Rβ and expression of other cellular signalling activities (Mills et al., 1991). It remains to be determined if the B cell IL-2 receptor is linked to the same cellular signalling effectors.

B lymphocytes respond to IL-2 by proliferation and by increased secretion of immunoglobulin. While it is unclear whether these two biological responses are mutually exclusive in a single lymphocyte, available data indicate that B cells may use the same cellular signalling pathway to couple cytokine binding to both responses (Tigges et al., 1989). The effect of IL-2 upon Ig secretion, particularly IgM, is explained by its capacity to elevate the biosynthesis of the J chain protein necessary for successful assembly and secretion of polymeric IgM. This effect is at the transcriptional level (Tigges et al., 1989) and is controlled by a *cis* promoter element, J_B, which is located at position -75 to -45 in the J chain gene promoter (Lansford et al., 1992). The J_B element is acted upon by a B cell-specific, IL-2-regulated *trans*-acting factor, NF-J_B. By analogy with the T cell system, the capacity of IL-2 to promote the growth of activated B cells may be explained by the induction of *ras*, and subsequent gene regulation by nuclear *trans*-activating factors such as serum response factor, c-*jun* and NF-κB (Downward et al., 1992). Indeed, in a transfected pre-B cell line, recent data have shown that IL-2 specifically induces the expression of the c-*fos* oncogene, an event which will be critical in facilitating expression of other genes which possess AP-1 sites in their promoter sequences (Hatakayama et al., 1992).

A further question which must be addressed is that of regulation of signals via the IL-2 receptor. Several mechanisms can exist to account for this, including rapid down-regulation of receptors following ligand binding by receptor mediated endocytosis. It is not unreasonable to expect that PTPases may be implicated in cytokine signalling regulation. Finally, it is noteworthy that in human B cells, IL-4 completely abrogates all of the effects of IL-2 upon GPI-signalling, cellular proliferation and Ig secretion (Eardley and Koshland, 1991; Jelinek and Lipsky, 1987; Galanaud et al., 1990; Lorente et al., 1990), indicating that the cellular signalling pathways activated by the two cytokines interact at some point. Molecular pharmacological explanations for the inhibition of IL-2-driven signalling by IL-4 remain to be fully elucidated.

IL-3, IL-5 and GM-CSF: shared receptor subunits

Early lymphoid cell progenitors are sustained in the bone marrow by haematopoietic factors such as GM-CSF and IL-3 (Kinashi et al., 1990), while eosinophil differentiation is regulated by IL-5 (Sanderson et al., 1989). Recent data have illustrated that IL-3, IL-5 and GM-CSF interact with receptors which have two components: an α chain which is specific for the individual cytokine, and a β chain which is shared by all three cytokine receptors (Tavernier et al., 1991). The α subunits of the IL-3, IL-5 and GM-CSF receptors have molecular masses of 70 kDa, 60 kDa and 80 kDa, respectively, while the common β subunit has a molecular mass of 120 kDa (reviewed in Miyajima et al., 1992). Transfection studies have illustrated that the β subunit is essential both for formation of high-affinity receptor complexes and for signal transduction (Metcalf et al., 1990).

IL-3, IL-5 and GM-CSF all induce PTK activities in cytokine-sensitive cells (Koyasu et al., 1988; Kanakura et al., 1990; Miyajima et al., 1992) although, in common with other HRS members, it is clear that neither the common β subunit nor the ligand-specific α subunits possess intrinsic tyrosine kinase domains. Since all three cytokines induce similar patterns of tyrosine phosphorylation of cellular proteins (Isfort and Ihle, 1990; Murata et al., 1990) it is possible that the common β subunit of the receptors associates with the same PTK and phosphorylates the same pool of substrates regardless of the ligand bound. The substrates for PTK activities associated with the IL-3 and GM-CSF receptors include c-*raf*-1 (Carroll et al., 1990; Kanakura et al., 1991), p21^{ras}(Satoh et al., 1991) and, in the case of IL-3, the β subunit of the IL-3 receptor itself (Sorensen et al., 1989).

There is also evidence that IL-3 (Linnekin and Farrar, 1990) and IL-5 (Murata et al., 1990) induce serine phosphorylation and, in the case of IL-3, that this is due to activation of PKC. Thus, IL-3 promotes a redistribution of PKC from the cytosol to the plasma membrane (Farrar et al., 1985), and drives cellular proliferation via a PKC-dependent mechanism (Whetton et al., 1988). Since IL-3 fails to promote inositol lipid hydrolysis, elevated PKC activity appears not to be a consequence of PLC action (Whetton et al., 1988); phosphatidylcholine may be the source of ligand-sensitive diacylglycerol in the IL-3 receptor system (Duronio et al., 1989).

IL-6: ligand-driven assembly of a functional receptor complex

IL-6 is a highly pleiotropic cytokine which elicits a range of responses in many different cell types, including B and T cells, hepatocytes, neural cells and fibroblasts (Wong and Clark, 1988; Kishimoto and Hirano, 1988; Van Snick, 1990). All sensitive cell types possess apparently identical IL-6 receptors. Little is known in detail about signal transduction mechanisms activated via the IL-6 receptor complex, but there are no reports of the cytokine stimulating lipid hydrolysis, PKC activation or Ca^{2+} mobilization. The IL-6 receptor complex comprises two distinct transmembrane glycoproteins, gp80 and gp130, both of which are members of the HRS. The gp80 component possesses a redundant Ig-like domain and binds ligand but is, by itself, unable to activate cellular signalling (Taga et al., 1989). However, the binding of IL-6 to gp80 promotes binding of gp80 to the second component of the receptor complex, gp130, and the transmission of cellular signalling information. Thus, gp130, which cannot bind IL-6, is the signal transduction element of the IL-6 receptor complex (Hibi et al., 1990), although it is unclear how these signals are transmitted given the lack of catalytic domains in the gp130 intracellular region. The sole signal for formation of the receptor complex capable of initiating second messenger generation is binding of IL-6, and this presumably is mediated allosterically via IL-6-induced exposure of an evanescent binding site for gp130 on the gp80 molecule. A final point of note in this system is that generation of a soluble, secreted form of gp80 can bind IL-6 and this complex of cytokine and soluble receptor retains its capacity to interact with gp130 and provide stimulatory signals to sensitive cells (Taga et al., 1989). Thus, the molecular motifs which participate in the interaction of gp80 and gp130 are located in the extracellular domains of the two glycoproteins. More interestingly, the soluble IL-6 receptor is an agonistic receptor, an apparently unique property amongst the soluble cytokine receptors since other well-characterized secreted cytokine receptors, for example IL-4 (Mosley et al., 1989) and IL-7 (Goodwin et al., 1990) behave as antagonistic soluble receptors.

Other cytokine receptors also possess gp130-like structures as part of their receptor elements. Thus, the receptor for Leukaemia Inhibitory Factor (LIF) displays sequence similarity to the gp130 element of the IL-6 receptor, particularly in its transmembrane and cytoplasmic domains (Gearing et al., 1991). The gp130 glycoprotein may be a member of a family of such molecules, all of which are involved in signal transduction via cytokine receptors. Sharing of a gp130-like element may account for cross-competition of IL-6 and LIF for ligand binding sites and their partially shared spectra of biological activities. This situation has a parallel with the shared β subunits in the IL-3/IL-5/GM-CSF model discussed above.

IL-7: PTK-coupled activation of PLC activity?

IL-7 is a stromal cell-derived cytokine (Henney, 1989) which is active upon B cell precursors (Namen et al., 1988) and certain immature T cell subsets (Chazen et al., 1989). However, the IL-7 receptor occupies an unusual position in this discussion inasmuch as there is little evidence to suggest a need for receptor dimerization. Thus, the sole component of the receptor characterized to date is a 75 kDa ligand-binding transmembrane glycoprotein which displays molecular motifs consistent with membership of the HRS. Studies on fetal thymocytes and leukaemic precursor B cell lines indicated that the IL-7 receptor was coupled to PtdInsP_2 hydrolysis via PTK-mediated activation of PLCγ1 (Uckun et al., 1991a,b) in a manner analogous to that reported for the T cell antigen-receptor complex. However, in striking contrast to these results, a recent report demonstrates that although the IL-7 receptor does indeed mediate tyrosine phosphorylation it is not coupled to PLC-γ1 phosphorylation or indeed any inositol phosphate generation or calcium mobilization in thymocytes, mature T cells and pre-pre-B cells (from a patient with acute lymphoblastic leukaemia) (Roifman et al., 1992). This major discrepancy may reflect differential coupling of the IL-7 receptor during differentiation and oncogenic transformation.

IL-4: species-specific signalling mechanisms?

The debate surrounding the biochemical mechanisms underlying IL-4 action on B lymphocytes has attracted perhaps the greatest controversy of all of the cytokines which regulate B cell development. IL-4 is a T cell-derived cytokine which elicits a range of effects upon B cell differentiation (Paul and Ohara, 1987). Thus, in the murine B cell system, it can sustain the growth of pre-B cells, promote early activation of quiescent B lymphocytes, which is manifest as increased expression of MHC class II antigens (Noelle et al., 1984; Roehm et al., 1984) and of low-affinity Fc_ϵ receptors (Hudak et al., 1987). Finally, IL-4 mediates directed isotype switching in mitogen-activated B cell blasts (Coffman et al., 1987; Lutzker et al., 1988; Rothman et al., 1988; Bergstedt-Lindqvist et al., 1988). A similar array of activities is evident in the human model, although increased levels of expression of membrane IgM and IgD are more pronounced, relative to murine models, at low doses of IL-4 (Rigley et al., 1991). This latter datum has implications for the existence of multiple IL-4 receptors.

The most compelling data currently available for signalling via the IL-4 receptor come from studies of human tonsillar B lymphocytes. In such cells, IL-4 promotes generation of InsP_3, diacylglycerol, and a rise in intracellular Ca^{2+} immediately after ligand binding. This is followed after several minutes by a sustained rise in intracellular cyclic AMP levels (Finney et al., 1990). In pharmacological mimicry experiments, the ability of IL-4 to stimulate CD23 expression, a marker of IL-4 action on human B cells, was achieved by a combination of a brief pulse with phorbol ester and ionomycin, followed by exposure to dibutyryl cyclic AMP. However, studies in murine B cells failed to find similar effects. Thus, stimulation of quiescent murine B cells with IL-4 fails to trigger inositol lipid hydrolysis and changes in intracellular Ca^{2+} levels, and also does not promote translocation of protein kinase C to the plasma membrane (Mizuguchi et al., 1986). Protein kinase activities do seem to be activated by IL-4 in B cells, however, as a 42 kDa protein of unknown identity is phosphorylated in isolated membranes in the presence of IL-4 (Justement et al., 1986; McGarvie and Cushley, 1989a). A similar protein kinase substrate has been reported in human tonsillar B cells, although the apparent molecular mass in this model is 38 kDa, slightly smaller than that reported in murine splenocytes (Finney et al., 1991).

The biochemistry of the IL-4 receptor also remains to be fully characterized. Initial experiments employing cross-linking of radioactive IL-4 to B cells suggested that, in murine and human models, IL-4 was principally associated with a 75 kDa cell surface component. However, similar studies of human B cells in other laboratories implicated a binding component of some 130–140 kDa (Galizzi et al., 1989). The IL-4 binding component was finally isolated by molecular cloning and shown to be of 140 kDa in the mature, fully glycosylated form in both murine (Mosley et al., 1989) and human (Idzerda et al., 1991) B cells. It remains as yet unclear if the 140 kDa component is the sole molecular species which makes up the IL-4 receptor. There are reports from cross-linking experiments which indicate the existence of species of 42 kDa and 110 kDa in murine B cells (Fernandez-Botran et al., 1990). Moreover, culture of murine B cells with IL-4 leads to the appearance of a 75 kDa tyrosine-phosphorylated protein in the membranes of such lymphocytes (McGarvie and Cushley, 1989b). No definitive cross-linking experiments have been reported for the human IL-4 receptor, but the crystallographic data for the growth hormone receptor (De Vos et al., 1992) and the dose-dependent nature of specific responses to IL-4 in human B cells (Rigley et al., 1991) suggests that other molecules are likely to be involved in modulation of the affinity and, possibly, component composition of the IL-4 receptor in B lymphocytes.

B lymphocytes respond to IL-4 in characteristic ways, one of which, in the murine model, is to greatly up-regulate the expression of class II MHC antigens (Noelle et al., 1984). This effect is not uniquely driven by IL-4 stimulation, and can also be elicited by gross stimulation with lipopolysaccharide and by anti-Ig treatment. However, in the case of class II antigen expression, the response is regulated at the molecular level by a specific DNA binding protein (NF-BRE) which interacts with a defined dodecanucleotide sequence element in the far upstream region of the promoter region of class II α chains (Boothby et al., 1989). The NF-BRE recognition element is distinct from the conserved W, X and Y boxes found in the 5 promoter region of all MHC class II genes. The precise biochemical requirements for activation of NF-BRE , and the cellular signalling pathways which deliver these, remain undefined.

ADHESION MOLECULES AND OTHER SURFACE STRUCTURES MEDIATING CELLULAR SIGNALLING IN B LYMPHOCYTES

The vast majority of data relating to cellular signalling in B cells has been derived from studies of the antigen and cytokine receptors. However, other cell surface structures, some with defined ligands and others with unknown ligands also initiate

signal transduction in B lymphocytes with consequences for activation, growth and differentiation of the B cell. Such structures include MHC antigens, adhesion molecules, receptors for complement components and Ig molecules, and members of the CD differentiation antigen family (Table 1). Signals delivered through these structures can influence the response of the B cell to antigen.

Adhesion molecules

The antigen receptors of B and T cells differ in one crucial respect; the B cell antigen receptor can bind antigen which is free in solution, and can do so with high affinity, whereas the T cell receptor cannot bind soluble antigen, requiring peptides derived from the intact antigen to be presented to it in association with MHC antigen; this binding is of several orders of magnitude lower affinity. In the case of T cells, adhesion molecules serve to promote the overall *avidity* of the multi-component recognition complex in order to facilitate specific binding of antigen.

The interaction of class II MHC antigen with CD4 could be regarded as a reaction between two adhesion molecules, although the interactions between CD2 on T cells and Leucocyte Functional Antigen-3 (LFA-3) on presenting cells (including B cells) would be considered a more classical pair of adhesion molecules, as would the ICAM-1 (CD54)–LFA-1 interaction (Figdor et al., 1990). These interactions have consequences for cellular signalling. Thus, it has been suggested that LFA-1 may be a Ca^{2+}-mobilizing receptor (Pardi et al., 1989), and it is well documented that anti-CD2 antibody alone triggers a range of cellular signalling processes including Ptd$InsP_2$ hydrolysis, mobilization of calcium, activation of PKC, PTK(s) and *ras*, all of which are generally believed to be activated via the CD3-$\zeta\eta$ complex (Downward et al., 1992). Moreover, simultaneous administration of anti-CD3 and anti-CD2 mAbs to T cells provides a powerful activatory signal, particularly if these are in the form of a bispecific antibody complex (Tutt et al., 1991). Thus, CD2 provides a further conduit for signal transduction in the T lymphocyte, and there is the possibility that the generation of the adhesion molecule pair sends a signal in both directions; in this example, the LFA-3^+ presenting cell could also be stimulated via LFA-3 as a consequence of the CD2–LFA-3 interaction. While the binding of antigen to the antigen receptor in B cells, even in low affinity and potentially polyreactive $mIgM^+/mIgD^+$ mature B cells, is of sufficiently high affinity to obviate the need for adhesion molecules in formation of a functional antigen recognition unit, B cells nonetheless possess a range of classical adhesion molecules which could potentially deliver cellular signals to the B cell during cognate recognition of thymus-dependent antigen. Additionally, the B lymphocyte possesses potentially unique adhesion molecule pairs, namely CD5/CD72 and B7/CD28 (De Franco, 1991) which could transduce activation signals to the B cell. In this regard, CD72 ligation is known to increase intracellular Ca^{2+} concentrations and to enhance proliferation induced by anti-Ig stimulation of B lymphocytes.

Cellular signalling via B lymphocyte differentiation antigens

B lymphocytes possess a range of cell surface differentiation CD (*C*luster of *D*ifferentiation) antigens, in addition to antigen and cytokine receptors, which are capable of transmitting information to the cell interior (reviewed in detail by Clark and Lane, 1991). It has already been noted that adhesion molecules have a role to play in signal transduction, and other molecules currently defined

Table 1 Summary of signals transmitted via B lymphocyte differentiation antigens

Surface antigen	Size (kDa)	Function	Signals	Effect of ligation on anti-Ig-stimulated events	References
CD19	95	Pan B cell marker	Possible increase in $[Ca^{2+}]_i$. PKC translocation to plasma membrane	Decreases c-*myc* mRNA levels	Clark and Ledbetter, 1989; Barrett et al., 1990; Rigley et al., 1991
CD20	35	Ca^{2+} channel	Increased c-*myc* and MHC class II via PKC activation. No Ptd$InsP_2$ or Ca^{2+} effects. Tyrosine kinase activation.	Stimulates quiescent B cells and inhibits activated B cells	Clark and Ledbetter, 1989; Clark and Lane, 1991; Brown et al., 1989
CD21	145	Receptor for C3d. Receptor for Epstein–Barr virus	Differentially phosphorylated in B cells. EBV causes Ptd$InsP_2$ hydrolysis and increases $[Ca^{2+}]_i$	Monomeric C3d lowers Ca^{2+} response, but anti-CD21 mAbs enhance.	Changelian and Fearon, 1986; Dugas et al., 1988; Tsokos et al., 1990
CD22	150	Adhesion molecule	Unknown	Boosts Ca^{2+} response	Knapp et al., 1989
CD23	45	Low-affinity IgE receptor. sCD23 has autocrine activity. Adhesion molecule	Increased $[Ca^{2+}]_i$ and Ptd$InsP_2$ hydrolysis		Kolb et al., 1990; Letellier et al., 1988; Kikutani et al., 1986
CD32	40	B cell Fc receptor for IgG	Increased $[Ca^{2+}]_i$ with anti-CD32	Inhibitory. Uncouples mIg from its G protein	Hunziker et al., 1990; Rigley et al., 1989
CD40	50	Adhesion molecule	Ser/Thr phosphorylation, but no Tyr phosphorylation	Enhances	Einfeld et al., 1988; Gruber et al., 1989
CD45	200	Protein tyrosine phosphatase		Blocks proliferation and increases $[Ca^{2+}]_i$	Tonks et al., 1988; Gruber et al., 1989; Ledbetter et al., 1988
CD72	85	Adhesion molecule	Increased $[Ca^{2+}]_i$	Enhances proliferation	Clark and Lane, 1991; Subbarao et al., 1988

by monoclonal antibodies also influence B cell activation, growth and differentiation. Many of these differentiation antigens influence, either positively or negatively, the cellular signalling pathways activated by anti-Ig stimulation of B cells. A brief mention of the signalling capabilities of some of the differentiation antigens is appropriate at this point; the list is not exhaustive (Table 1). The elucidation of cellular signalling via such molecules is in its infancy, and depends upon use of mAbs to these structures since the normal ligands for many CD antigens remain undefined.

However, many CD antigens are receptors for known ligands. Thus, CD21 is the receptor for the complement component C3d and for Epstein–Barr virus, while CD23 functions as a low affinity receptor for IgE. Other CD structures, however, do not have defined ligands, and much of the information regarding their signalling functions comes from studies using mAbs directed against the structures. Thus, stimulation of B cells with anti-CD19 mAbs causes increases in intracellular Ca^{2+} levels and a translocation of protein kinase C to the plasma membrane (Clark and Ledbetter, 1989), although other groups fail to note Ca^{2+} mobilization following anti-CD19 stimulation (Rigley et al., 1991). In addition, while anti-CD20 mAbs fail to influence intracellular Ca^{2+} levels (Lane et al., 1991; Clark and Lane, 1991), both anti-CD19 and anti-CD20 mAbs can influence the signals transmitted via the antigen receptor, although the precise nature of the modulatory effect can be determined by the activation status of the B cells (Clark and Ledbetter, 1989; Brown et al., 1989; Barrett et al., 1990; Rigley et al., 1991).

Analysis of the data from the CD21 system suggest that due caution should be applied when interpreting data from signalling experiments in complex cellular systems using mAbs as stimulatory agents. Thus, anti-CD21 mAbs fail to mobilize intracellular Ca^{2+}(Dugas et al., 1988), whereas Epstein–Barr virus (one of the physiological ligands) does so (Carter et al., 1988). Moreover, monomeric C3d inhibits anti-Ig stimulated Ca^{2+} levels, but anti-CD21 mAbs enhance this response (Tsokos et al., 1990). A final point of note with respect to CD21 is the recent finding that CD23 binds to this differentiation antigen (Aubry et al., 1992). The reaction can be blocked by some, but not all, anti-CD21 mAbs, indicating that CD21 functions as a receptor for CD23 as well as for Epstein–Barr virus and C3d. This also raises the possibility of signalling in both directions as a result of CD21–CD23 interaction as both these differentiation antigens are known to drive Ca^{2+} mobilization following ligation. The interaction of the CD5 and CD72 molecules provides another example of potential bi-directional signalling.

One particular B cell differentiation antigen which has attracted particular attention is CD40. Ligation of CD40 by antibody leads to a number of effects on B cells and influences signals delivered via other receptors (notably the IL-4 and antigen receptors). Thus, use of mAbs to cross-link CD40 on the B cell surface leads to elevated release of sCD23 (Cairns et al., 1988) and also promotes synthesis and secretion of IL-6 (Clark and Shu, 1990). Use of anti-CD40 and IL-4 in conjunction with $CD32^+$ adherent cells leads to the establishment of long-term B cell lines from normal primary human B cells (Banchereau et al., 1991). Studies of cellular signalling via CD40 suggest a role for PTK activities (Uckun et al., 1991c) and, indeed, CD40 shows some sequence similarity to members of the Nerve Growth Factor family. In germinal centre cells, ligation of CD40 is one of the signals required to allow centrocytes to escape from apoptosis, an effect mediated via induction of *bcl*-2 expression in the centrocytes (Liu et al., 1992). The CD40 ligand has recently been cloned, and is a 39 kDa structure expressed on activated T lymphocytes. Anti-39 kDa mAbs block the capacity of this structure to activate B cells for growth and differentiation, showing that this molecule has a critical role in B cell activation (Noelle et al., 1992).

RECEPTOR CROSS-TALK AND REGULATION OF SIGNAL TRANSDUCTION IN B LYMPHOCYTE PROLIFERATION AND DIFFERENTIATION

The foregoing discussion illustrates that the B lymphocyte provides a range of ligand receptor systems which are interesting and worthy of investigation as individual models. However, there are numerous interactions between receptor systems in B cells which are particularly important for the insights which they may yield into mechanisms of receptor desensitization and in the positive and negative interaction of cellular signalling networks within the B cell. Some examples of interacting signal transduction systems are now discussed.

Homologous and heterologous desensitization of antigen receptors

Perturbation of the antigen receptor on the B cell is the critical event in the initiation of clonal expansion; all subsequent ligands deliver essentially regulatory signals. The cellular signalling systems activated by stimulation of the antigen receptor have been documented above. However, it has been well-documented that following ligand binding, the antigen receptor, like many other receptors, enters a period where it is refractory to further stimulation. In the case of a mature $mIgM^+/mIgD^+$ B lymphocyte, stimulation of the cell with anti-μ-specific reagents causes not only desensitization of mIgM-containing antigen receptors, but also results in the desensitization of the heterologous, mIgD antigen receptor complexes; the same is true when anti-δ reagents are employed as primary stimulus (Klaus et al., 1985; Cambier et al., 1988). Inositol phospholipid signalling via the mIgM and mIgD receptors appear to be regulated by a common form of G-protein, and desensitization appears to be at the level of this G-protein–PLC coupling (Harnett et al., 1989). Neither homologous nor heterologous desensitization processes are permanent, and the B cell regains its capacity to mobilize Ca^{2+} in response to anti-Ig after 8–20 h of initial stimulation (Cambier et al., 1988; Harnett et al., 1989).

Stimulation of membrane Ig leads not only to homologous and heterologous desensitization of mIg molecules, but also influences the generation of signals via other cell surface receptors. Thus, in human B cells, both CD21 (Carter et al., 1988) and, more controversially (Uckun et al., 1988; Rigley et al., 1989), CD19 mobilize Ca^{2+} when stimulated by mAbs. Exposure of B cells to anti-μ or anti-δ antibodies prevents subsequent mobilization of Ca^{2+} in response to anti-CD19 or anti-CD21 stimulation (Rijkers et al., 1990).

Antigen, Fc and IL-4 receptors: three-way receptor cross-talk

In an on-going immune response, activated B cells are subject to negative regulation by circulating immunoglobulin, which is important in feedback inhibition of the antibody response. In B lymphocytes, such negative feedback regulation of activation and antibody secretion is mediated by the 40 kDa Fc receptor, CD32 (Hunziker et al., 1990). Occupation of the B cell CD32 structure sends a profoundly negative signal to the B lymphocyte rendering it refractory to signals received via the antigen receptor and resulting in abortive activation upon antigenic stimulation (Klaus et al., 1984). However, the negative influence of occupied CD32 can be overcome in B cells by IL-4 (O'Garra et al., 1987). Thus, exposure of B cells to IL-4 prior to treatment with intact

anti-Ig molecules prevents the abortive activation caused by simultaneous occupation of antigen and Fc receptors by the intact IgG antibodies. Thus, IL-4 over-rides and/or negates the signal delivered via CD32, or maintains a key element of the antigen receptor signal transduction pathway in an "active" condition refractory to the negative influence of occupied CD32.

The biochemical mechanism underlying the FcR-dependent inhibition of mIg signalling has been at least partially elucidated. Thus, whilst $F_{(ab)2}$ fragments of anti-Ig provoke a sustained hydrolysis of Ptd$InsP_2$, intact anti-Ig causes only a transient release of $InsP_3$. Moreover, intact anti-Ig, which co-crosslinks mIg and Fc receptors, profoundly inhibits the release of $InsP_3$ provoked by the mitogenic $F_{(ab)2}$ fragments. Thus, the effect of occupation of CD32 is to reduce PLC activity, such that little Ca^{2+} can be mobilized and activation is abortive (Bijsterbosch and Klaus, 1985). Mechanistically, this effect is explained in terms of the functional coupling of the antigen receptor complex to its G protein (Rigley et al., 1989). In permeabilized B cells, $F_{(ab)2}$ anti-Ig reagents plus GTPγS can reconstitute the mIg-mediated inositol lipid hydrolysis observed in intact cells. Co-stimulation of these permeabilized B cells with intact anti-Ig antibodies abrogates the functional reconstitution, but does not disrupt the GTPγS-induced PLC activity (Rigley et al., 1989). These data indicate that the occupied FcR mediates its effect by disrupting the function of the B cell antigen receptor complex at a point proximal to the G protein. Thus, whilst the G protein itself is functionally linked to PLC, as demonstrated by the lack of effect of anti-Ig upon GTPγS-induced lipid hydrolysis, the G protein itself appears to be uncoupled from the antigen receptor (reduction of GTPγS plus anti-Ig response to that of GTPγS alone). Thus, the G protein can transmit signalling information, but cannot receive stimulatory input from the B cell antigen receptor complex (Rigley et al., 1989). Although the mechanism leading to this uncoupling is unknown, one possibility is that engaging CD32 could disrupt receptor–Gp contact by modifying the receptor contact site with Gp, perhaps by phosphorylation (Rall and Harris, 1987; Sullivan et al., 1987). Alternatively, it has been shown previously that capping of sIg also leads to co-capping of FcR, suggesting that the uncoupling might be brought about by physical dissociation, perhaps by disrupting the sIg/accessory transducing molecules complex. IL-4 reverses the capacity of FcR to inhibit stimulatory signals delivered via the antigen receptor (O'Garra et al., 1987), and although the molecular basis for this effect has not been elucidated, it is clear that IL-4 does not overcome the inhibition of Ptd$InsP_2$ hydrolysis in murine B cells (O'Garra et al., 1987).

Receptor cross-talk mechanisms underlying T cell-dependent cell activation

At present, the molecular mechanisms underlying the complex interactions of signals generated by mIg, IL-4R (and other cytokine receptors such as IL-5R in the mouse), CD40, and class II molecules which lead to B cell proliferation and differentiation are poorly understood (Cambier and Lehmann, 1989; Cambier et al., 1991). However, although it has not yet been possible to assign a particular signalling pathway to the IL-4 receptors on murine B cells, some progress has been made towards understanding cross-talk mechanisms underlying the interactions between the antigen and IL-4 receptors during the priming phase (Harnett et al., 1991). Thus, although IL-4 does not appear to induce the release of $InsP_3$, Ca^{2+} mobilization, PKC translocation (Justement et al., 1986; Mizuguchi et al., 1986), or indeed to modify signalling via the phosphoinositide pathway induced by ligation of the sIg receptors in murine B cells (Klaus and Harnett, 1990), recent evidence demonstrates that IL-4 synergizes with non-mitogenic concentrations of anti-Ig to provoke translocation of PKC from the cytosol to the plasma membrane (Harnett et al., 1991). Thus, the lymphokine upregulates PKC levels and activity and also acts to prevent PKC downregulation in B cells (Harnett et al., 1991). These data therefore suggest that signals generated via IL-4 receptors potentiate and/or prolong sIg-induced PKC activation and may provide a biochemical basis for explaining how IL-4 and anti-Ig synergize to induce B cell activation. The proposal that strong, prolonged PKC signals are sufficient to induce DNA synthesis in murine B cells is also likely to be consistent with the other models (Cambier and Lehmann, 1989; Cambier et al., 1991) of T cell-dependent B cell activation: Cambier's group were initially puzzled by the finding that mIg, IL-4R and Class II ligation synergised to induce B cell entry to S phase as earlier studies reported that (i) ligation of Class II molecules could induce cyclic AMP elevation and inhibit mitogen-driven proliferation, and (ii) it was widely accepted that elevation of cyclic AMP levels would be antagonistic for lymphocyte proliferation (Forsgren et al., 1984; Cambier et al., 1987; Cohen and Rothstein, 1989). This paradox now appears to have been resolved by the recent finding that cross-linking of Class II molecules on murine B cells pretreated with both IL-4 and anti-Ig generates an inositol phospholipid (and presumably PKC) response rather than the cyclic AMP response observed in resting cells. Furthermore, the inositol phospholipid response resulting from crosslinking Class II on IL-4/anti-Ig "primed" cells is of greater magnitude than that observed when anti-Ig alone is employed as primary stimulus (Cambier et al., 1991). Although this model of murine B cell activation now appears to be highly dependent on calcium and PKC signals, it may not be prudent to totally discard a role for Class II-mediated cyclic AMP signals at some stage in this process as the likely signals generated by mIg, IL-4R and Class II molecules on resting B cells are reminiscent of the PKC/cyclic AMP response associated with the IL-4R on human B lymphocytes (Finney et al., 1990; Rigley and Harnett, 1990).

CONCLUSION AND FUTURE PERSPECTIVES

Great advances have been made in the understanding of the structures and functions of receptors for antigen and cytokines on B lymphocytes, and the biological consequences of the signals delivered via these receptors. It is clear that the antigen receptors of both T and B lymphocytes are multi-component assemblies comprising both clonotypic components and conserved elements involved in complex assembly, transport and signal transduction. Many cytokine receptors, particularly those of the haematopoietin receptor superfamily, also appear to be multi-component complexes, but in this instance additional interest is provided by the fact that assembly of functional complexes may be driven by ligand (e.g., IL-6 and IL-2 receptors), and a novel receptor–ligand stoichiometry (2:1) seems to prevail in these systems.

Interesting questions do remain to be resolved, including the precise mechanisms via which the antigen receptors are coupled to the PLC signalling effector. Is the critical coupling event mediated via a PTK, G protein or both? Evidence to support either mechanism is available in abundance, but it is entirely possible that both systems may operate, to greater or lesser extents, in B cells at different stages of differentiation or depending upon other stimuli which the cells may have previously met. It could be argued that virgin and memory B cells have the capacity to encounter and respond to antigen in qualitatively different ways. For example, soluble anti-Ig reagents are generally

adequate in induction of activation of mIgM$^+$/mIgD^{++} B cells, while the same reagents fail to rescue centrocytic cells from apoptosis, anti-Ig immobilized on Sepharose being mandatory to achieve this outcome. Thus, virgin cells respond to soluble antigen while recently formed memory B cells (of non-mIgM/mIgD isotypes) require to encounter insoluble antigen, at least on the first occasion, in order to mount a positive response. Given that there are little or no apparent differences in the complement of receptor complex components, might this reflect subtle differences in the coupling of original and somatically mutated B cell antigen receptors?

Good experimental models for the study of B lymphocyte activation processes are not abundant. Cell lines are transformed and are therefore, by definition, not quiescent. Moreover, the transformation process may involve expression or over-expression of cellular proto-oncogenes which ablate a normal cellular regulatory function, thereby compromising the normal physiological linkage of a receptor to its signalling effector system(s). Abnormal expression of any kinase (e.g. *lyn*, *raf*), GTP binding protein (e.g. *ras*) or transcription factor (e.g. *fos*) could easily bias the activation system used by a particular receptor in a transformed cell. Therefore, data from normal B cell systems and those from B cell lines should not be regarded as strictly comparable.

Moreover, while lessons can be learned from data in the T cell receptor system, it is worth considering that there is no *a priori* reason why the antigen receptors of the two lymphocyte types should be linked to their signalling effectors by the same coupling intermediates. Thus, clonotypic T cell receptors experience antigen at very low affinity (relative to B lymphocyte receptors) and the functional activation signal may result from the net avidity of multiple receptor–ligand systems rather than simply stimulation of the TcR itself. In this context, the relevance of use of anti-TcR (or CD3ϵ) mAbs as a stimulus to the physiological state should be regarded cautiously; for example, the concentration of anti-TcR reagents used to drive PtdInsP_2 hydrolysis frequently induces cell death rather than activation in some T cell models (Cherwinski et al., 1992).

While several of the downstream signalling events associated with many cytokine receptors are well defined, including PTK activation, c-*raf* and p21^{ras} activation, the initial coupling events are less well understood. Moreover, interpretation of data from cytokine systems faces the additional complication of receptors of different affinities. Taking human IL-4 as a particular example, low doses of cytokine promote elevation of membrane Ig levels, while higher doses appear to drive directed isotype switching; IL-4 causes inositol lipid hydrolysis and cyclic AMP production in human B cells but it is not known which of these two signalling pathways, *if either*, is linked to the biological responses observed. The same questions arise for other cytokines where multiple cellular responses can be elicited by a single cytokine. Since B cells are likely to encounter high local doses of cytokines within secondary lymphoid organs during an on-going response to antigen, it may be that responses to ligation of low affinity receptors will prove to be more physiologically relevant than at first thought.

It is clear than B lymphocytes can mount characteristically distinct responses to antigen and/or cytokine stimulation depending upon their differentiation status. In a few cases, such as the expression of *egr*-1 in mature B cells being correlated with a positive response to stimulation via mIg, the basis for differentiation state-dependent responses is beginning to be understood. However, data of this kind suggest that substrates for signalling effector systems linked to many B cell receptors may differ in a differentiation stage-dependent manner, and it is the range of substrates available at the time of contact with ligand (antigen, cytokine or adhesion structure) which determines the ultimate biological response of the B lymphocyte. The identification and characterization of such putative substrates and their cellular functions awaits elucidation, and will provide the next step forward in our understanding of regulation of cellular signalling systems in the B lymphocyte system.

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REFERENCES

Ales-Martinez, J. E., Cuende, E., Martinez, A, C., Parkhouse, R. M. E., Pezzi, L. and Scott, D. W. (1991) Immunol. Today **12**, 201–205

Alexander, D. R. (1990) The New Biologist **2**, 1049–1062

Alt, F. W., Bothwell, A. L.M, Knapp, M., Siden, E., Mather, E., Koshland, M. and Baltimore, D. (1981) Cell **20**, 293–301

Arima, N., Kamio, M., Imada, K., Hari, T., Hattori, T., Tsudo, M., Okuma, M. and Uchiyama, T. (1992) J. Exp. Med. **176**, 1265–1272

Aubry, J.-P., Pochon, S., Graber, P., Jansen, K. U. and Bonnefoy, J.-Y. (1992) Nature (London) **358**, 505–507

Banchereau, J., De Paoli, A., Vallé, A., Garcia, E. and Rousset, F. (1991) Science **251**, 70–72

Barrett, T. S., Shu, G. L., Draves, K. E., Pezzutto, A. and Clark, E. A. (1990) Eur. J. Immunol. **20**, 1053–1059

Bazan, H. F. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 6934–6938

Bergstedt-Lindqvist, S., Moon, H.-B., Persson, U., Möller, G., Heusser, C. and Severinson, E. (1988) Eur. J. Immunol. **18**, 1073–1077

Bijsterbosch, M. K. and Klaus, G. G. B. (1985) J. Exp. Med. **162**, 1825–1836

Bijsterbosch, M., Meade, J. C., Turner, G. A. and Klaus, G. G. B. (1985) Cell **41**, 999–1005

Boothby, M., Gravallesse, E., Liou, H.-C. and Glimcher, L. H. (1989) Science **242**, 1559–1562

Brian, A. A. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 564–570

Brown, A. N., Thurstan, S. M. and Callard, R. E. (1989) in Leukocyte Typing IV; White Cell Differentiation Antigens, pp. 203–205, Oxford University Press, Oxford

Burke, T., Pollok, K., Cushley, W. and Snow, E. C. (1989) Mol. Immunol. **26**, 1095–1104

Burkhardt, A. L., Brunswick, M., Bolen, J. B. and Mond, J. J. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 7410–7414

Burnet, M. F. (1959) The Clonal Selection Theory of Acquired Immunity, Vanderbilt University Press

Burton, J., Goldman, C. K., Rao, P., Moos, M. and Waldmann, T. A. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 7329–7333

Butcher, R. D. J. and Cushley, W. (1991) Immunology **74**, 511–518

Butcher, R. D. J., McGarvie, G. M. and Cushley, W. (1990) Immunology **69**, 57–64

Cairns, J. L., Flores-Romo, L., Millsum, M. J., Guy, G. R., Gillis, S., Ledbetter, J. A. and Gordon, J. (1988) Eur. J. Immunol. **18**, 349–356

Callard, R. E., Smith, S. H., Shields, J. G. and Levinsky, R. J. (1986) Eur. J. Immunol. **16**, 1037–1042

Callard, R. E. (1990) Cytokines and B Lymphocytes, Academic Press, London

Cambier, J. C. and Campbell, K. S. (1990) Semin. Immunol. **2**, 139–149

Cambier, J. C. and Lehman, K. R. (1989) J. Exp. Med. **170**, 877–886

Cambier, J. C., Newell, M. K., Justement, L. B., McGuire, J. C., Leach, K. L. and Chen, Z. Z. (1987) Nature (London) **327**, 629–632

Cambier, J. C., Chen, Z. Z., Pasternak, J., Ransom, J., Sandoval, V. and Pickles, H. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 6493–6497

Cambier, J. C., Morrison, D. C., Chien, M. M. and Lehmann, K. R. (1991) J. Immunol. **146**, 2075–2082

Campbell, K. S. and Cambier, J. C. (1990) EMBO J. **9**, 441–448

Campbell, M. A. and Sefton, B. (1990) EMBO J. **9**, 2125–2131

Carroll, M. P., Clark-Lewis, I., Rapp, U. R. and May, W. S. (1990) J. Biol. Chem. **265**, 19812–19817

Carter, R. H., Park, D. J., Rhee, S. G. and Fearon, D. T. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 2745–2749

Casillas, A., Hanekom, C., Williams, K., Katz, R. and Nel, A. (1991) J. Biol. Chem. **266**, 19088–19094

Changelian, P. S. and Fearon, D. T. (1986) J. Exp. Med. **163**, 101–115

Chazen, G. D., Pereira, G. M. B., LeGros, G., Gillis, S. and Shevach, E. M. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 5923–5927

Chen, J., Stall, A. M., Herzenberg, L. A. and Herzenberg, L. A. (1990) EMBO J. **9**, 2117–2124

Cherwinski, H. M., Semenuk, G. T. and Ransom, J. T. (1992) J. Immunol. **148**, 2996–3003

Clark, E. A. and Lane, P. J. L. (1991) Annu. Rev. Immunol. **9**, 97–127

Clark, E. A. and Ledbetter, J. A. (1989) Adv. Cancer Res. **52**, 81–149

Clark, E. A. and Shu, G. L. (1990) J. Immunol. **145**, 1400–1411
Coffman, R. L., Ohara, J., Bond, M. W., Carty, J., Zlotnik, A. and Paul, W. E. (1986) J. Immunol. **136**, 4538–4541
Cohen, D. P. and Rothstein, T. L. (1989) Cell. Immunol. **121**, 113–124
Coleclough, C., Perry, R. P., Karjalainen, K. and Weigert, M. (1981) Nature (London) **290**, 372–378
Cosman, D., Lyman, S. D., Idzerda, R. L., Beckmann, M. P., Park, L. S., Goodwin, R. G. and March, C. J. (1990) Trends Biochem. Sci. **15**, 265–269
Cunningham, B. C., Ultsch, M., De Vos, A. M., Mulkerrin, M. G., Clauser, K. R. and Wells, J. A. (1991) Science **254**, 821–825
Curtis, B. M., Widmer, M. B., DeRoos, P. and Qwarnstrom, E. E. (1990) J. Immunol. **144**, 1295–1303
D'Andrea, A. D., Fasman, G. D. and Lodish, H. F. (1989) Cell **58**, 1023–1024
Dasgupta, J. D., Granja, C., Druker, B., Lin, L.-L., Yunis, E. J. and Relias, V. (1992) J. Exp. Med. **175**, 285–288
De Franco, A. L. (1991) Nature (London) **353**, 603–604
De Vos, A. M., Ultsch, A. and Kossiakoff, A. A. (1992) Science **255**, 306–312
Dewald, B., Thelen, B. and Baggiolini, M. (1988) J. Biol. Chem. **263**, 16179–16184
Dinarello, C. A., Clark, B. D., Puren, A. J., Savage, N. and Rosoff, P. M. (1989) Immunol. Today **10**, 49–51
Downward, J., Graves, J. D., Warne, P. H., Rayter, S. and Cantrell, D. A. (1990) Nature (London) **346**, 719–723
Downward, J., Graves, J. and Cantrell, D. A. (1992) Immunol. Today **13**, 89–92
Dugas, B., Delfraissy, J. F., Calenda, A., Peuchmaur, M., Wallon, C., Rannou, M. T. and Galanaud, P. (1988) J. Immunol. **141**, 4344–4351
Duronio, V., Nip, L. and Pelch, S. L. (1989) Biochem. Biophys. Res. Commun. **164**, 804–808
Eardley, D. D. and Koshland, M. E. (1991) Science **251**, 78–81
Einfeld, D. A., Brown, J. P., Valentine, M. A., Clark, E. A. and Ledbetter, J. A. (1988) EMBO J. **7**, 711–717
Farrar, W. L. and Anderson, W. B. (1985) Nature (London) **315**, 233–234
Farrar, W. L., Thomas, T. P. and Anderson, W.B (1985) Nature (London) **315**, 235–237
Fernandez-Botran, R., Uhr, J. W. and Vitetta, E. S. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 4235–4239
Figdor, C. G., van Kooyk, Y. and Keizer, G. D. (1990) Immunol. Today **11**, 277–283
Finkel, T. H., Kubo, R. T. and Cambier, J. C. (1991) Immunol. Today **12**, 79–85
Finney, M., Guy, G., Michell, R. H., Gordon, J., Dugas, B., Rigley, K. P. and Callard, R. E. (1990) Eur. J. Immunol. **20**, 151–156
Finney, M. J., Michell, R. H., Gillis, S. and Gordon, J. (1991) Biochem. Soc. Trans. **19**, 287–291
Forsgren, S., Pobor, G., Coutinho, A. and Pierres, M. (1984) J. Immunol. **133**, 2104–2110
Frank, S. J., Samelson, L. E. and Klausner, R. D. (1990) Semin. Immunol. **2**, 89–98
Galanaud, P., Karray, D. S. and Lorente, L. L. (1990) Eur. Cytokine Network **1**, 57–64
Galizzi, J.-P., Castle, B., Djossou, O., Harada, N., Cabrillat, H., Yahia, S. A., Barrett, R., Howard, M. and Banchereau, J. (1990) J. Biol. Chem. **265**, 439–444
Gearing, D. P., Thut, C. J., Vanden Bos, T., Gimpel, S. D., Delaney, P. B., King, J., Price, V., Cosman, D. and Beckmann, M. P. (1991) EMBO J. **10**, 2839–2848
Germain, R. and Hendrix, L. A. (1991) Nature (London) **353**, 134–139
Gold, M. R., Jakway, J. P. and DeFranco, A. L. (1987) J. Immunol. **139**, 3604–3613
Gold, M. R., Law, D. and DeFranco, A. L. (1990) Nature (London) **345**, 810–813
Gold, M. R., Matsuuchi, L., Kelly, R.B and De Franco, A. L. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 3436–3440
Gold. M. R., Sanghera, J. S., Stewart, J. and Pelech, S. L. (1992) Biochem. J. **287**, 269–276
Goodnow, C. C., Crosbie, J., Adelstein, S., Lavoie, T. B., Smith-Gill, S., Brink, R. A., Pritchard-Bristoe, H., Wotherspoon, J. S., Loblay, R. H., Raphael, K., Trent, R. J. and Basten, A. (1988) Nature (London) **334**, 676–682
Goodwin, R. G., Friend, D., Ziegler, S. F., Jerzy, R., Falk, B. A., Gimpel, S., Cosman, D., Dower, S. K., March, C. J., Namen, A. E. and Park, L. S. (1990) Cell **60**, 941–951
Graves, J. and Cantrell, D. (1991) J. Immunol. **146**, 2102–2107
Greene, W. C., Bohnlein, E. and Ballard, D. W. (1989) Immunol. Today **10**, 272–278
Grenfell, S., Smithers, N., Miller, K. and Solari, R. (1989) Biochem. J. **264**, 813–822
Gruber, M. F., Bjorndahl, J. M., Nakamura, S. and Fu, S. M. (1989) J. Immunol. **142**, 4142–4152
Grupp, S. A., Snow, E. C. and Harmony, J. A. K. (1987) Cell. Immunol. **109**, 181–191
Harnett, M. M. and Klaus, G. G. B. (1988) J. Immunol. **140**, 3135–3139
Harnett, M. M., Holman, M. and Klaus, G. G. B. (1989) Eur. J. Immunol. **19**, 1933–1939
Harnett, M. M., Holman, M. J. and Klaus, G. G. B. (1991) J. Immunol. **147**, 3831–3835
Hasbold, J. and Klaus, G. G. B. (1990) Eur. J. Immunol. **20**, 1685–1690
Hatakayama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyusaka, M. and Taniguchi, T. (1989) Science **244**, 551–556
Hatakayama, M., Kono, T., Kobayashi, N., Kawahara, A., Levin, S. D., Perlmutter, R. M. and Taniguchi, T. (1991) Science **252**, 1523–1528
Hatakayama, M., Kawahara, A., Mori, H., Shibuya, H. and Taniguchi, T. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 2022–2026
Hempel, W. M. and DeFranco, A. L. (1991) J. Immunol. **146**, 3713–3720
Henderson, S., Rowe, M., Gregory, C., Croom-Carter, C., Wang, F., Longnecker, R., Kieff, E. and Rickinson, A. (1991) Cell **65**, 1107–1115
Henney, C. S. (1989) Immunol. Today **10**, 170–173
Hermanson, G. G., Eisenberg, D., Kincade, P. W. and Wall, R. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 6890–6894
Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T. and Kishimoto, T. (1990) Cell **63**, 1149–1157
Hirano, T., Taga, T., Nakano, N., Yasukawa, K., Kashiwamura, S., Shimizu, K., Nakajima, K., Pyun, K. H. and Kishimoto, T. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 5490–5494
Hodgkin, P. D., Yamashita, L. C., Coffman, R. L. and Kehry, M.R (1990) J. Immunol. **145**, 2025–2034
Holmes, W. E., Lee, J., Kuang, W.-J., Rice, J. C. and Wood, W. I. (1991) Science **253**, 1278–1280
Hombach, J., Sablitzky, F., Rajewsky, K. and Reth, M. (1988) J. Exp. Med. **167**, 652–657
Hombach, J., Tsubata, T., Leclercq, L., Stappert, H. and Reth, M. (1990) Nature (London) **343**, 760–762
Howard, M., Farrar, J., Hilfiker, M., Johnson, B., Takatsu, K., Hamaoka, T. and Paul, W. E. (1982) J. Exp. Med. **155**, 914–924
Hudak, S., Gollnick, S. O., Conrad, D. H. and Kehry, M. R. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 4606–4610
Hunziker, W., Koch, T., Whitney, S. A. and Mellman, I. (1990) Nature (London) **345**, 628–632
Idzerda, R. L., March, C. J., Mosley, B., Lyman, S. D., Vanden Bos, T., Gimpel, S. D., Din, W. S., Grabstein, K. H., Widmer, M. B., Park, L. S., Cosman, D. and Beckmann, M. P. (1990) J. Exp. Med. **171**, 861–873
Isfort, R. J. and Ihle, J.N (1990) Growth Factors **2**, 213–220
Jelinek, D. F. and Lipsky, P. E. (1987) J. Immunol. **141**, 164–173
Jelinek, D. F., Slawski, J. and Lipsky, P. (1986) Eur. J. Immunol. **16**, 925–932
June, C. H., Fletcher, M. C., Ledbetter, J. A., Schieven, G. L., Siegel, J. N., Phillips, A. F. and Samelson, L. E. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 7722–7726
Justement, L. B., Chan, Z., Harris, L., Ransom, J., Sandoval, V., Smith, C., Rennick, D., Roehm, N. and Cambier, J. C. (1986) J. Immunol. **137**, 456–464
Justement, L. B., Campbell, K. S., Chien, N. C. and Cambier, J. C. (1991) Science **252**, 1839–1842
Kanakura, Y., Druker, B., Cannistra, S. A., Furukawa, Y., Torimoto, Y. and Griffin, J. D. (1990) Blood **76**, 706–715
Kanakura, Y., Druker, B., Wood, K. W., Mamon, H. J., Okuda, K., Roberts, T. M. and Griffin, J. D. (1991) Blood **77**, 243–248
Kikutani, H., Suemura, M., Owaki, H., Nakamura, H., Sato, R., Yamasaki, K., Barsumian, E. L., Hardy, R. R. and Kishimoto, T. (1986) J. Exp. Med. **164**, 1455–1469
Kinashi, T., Tashiro, K., Lee, K. H., Inaba, K., Toyama, K., Palacios, R. and Honjo, T. (1990) Philos. Trans. R. Soc. London Ser. B **327**, 117–125
Kishimoto, T, and Hirano, T. (1988) Bioessays **9**, 11–15
Klaus, G. G. B. and Harnett, M. M. (1990) Eur. J. Immunol. **20**, 2301–2307
Klaus, G. G. B., Hawrylowicz, C. M., Holman, M. J. and Keeler, M. D. (1984) Immunology **53**, 693–698
Klaus, G. G. B., Bijsterbosch, M. and Parkhouse, R. M. E. (1985) Immunology **54**, 677–683
Knapp, W., Dorken, B., Gilks, W. R., Rieber, E. P., Schmidt, R. E., Stein, H. and van dem Borne, A. E. G. (1989) Leukocyte Typing IV: White Cell Differentiation Antigens, Oxford University Press, Oxford
Kolb, J.-P., Renard, D., Dugas, B., Genot, E., Petit-Koskas, E., Sarfati, M., Delespesse, G. and Poggioli, J. (1990) J. Immunol. **145**, 429–437
Koretzky, G. A., Picus, J., Thomas, M. L. and Weiss, A. (1990) Nature (London) **346**, 66–68
Koyasu, S., Tojo, A., Miyajima, A., Akiyama, T., Kasuga, M., Urabe, A., Schreurs, J., Arai, K., Takaku, F. and Yahara, I. (1987) EMBO J. **6**, 3979–3984
Lane, P. J., Ledbetter, J. A., McConnell, F. M., Draves, K., Deans, J., Schieven, G. and Clark, E. A. (1991) J. Immunol. **146**, 715–722
Lansford, R. D., McFadden, H. J., Siu, S. T., Cox, J. S., Cann, G. M. and Koshland, M. E. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 5966–5970
Letellier, M., Sarfati, M. and Delespesse, G. (1989) Mol. Immunol. **26**, 1105–1112
Liang, M. and Garrison, J. C. (1991) J. Biol. Chem. **266**, 13342–13349
Linnekin, D. and Farrar, W. L. (1990) Biochem. J. **271**, 317–324
Liu, Y.-J., Joshua, D. E., Williams, G. T., Smith, C. A., Gordon, J. and MacLennan, I. C. M. (1989) Nature (London) **342**, 929–931
Liu, Y.-J., Cairns, J. A., Holder, M., Abbot, S. D., Jansen, K. H., Bonnefoy, J.-Y., Gordon, J. and MacLennan, I. C. M. (1991a) Eur. J. Immunol. **21**, 1107–1114
Liu, Y.-J., Mason, D. Y., Johnson, G. D., Abbot, S., Gregory, C. D., Hardie, D. L., Gordon, J. and MacLennan, I. C. M. (1991b) Eur. J. Immunol. **21**, 1905–1910
Liu, Y.-J., Johnson, G. D., Gordon, J. and MacLennan, I. C. M. (1992) Immunol. Today **13**, 17–21

Lorente, L. L., Mitjavila, F., Crevon, M.-C. and Galanaud, P. (1990) Eur. J. Immunol. **20**, 1887–1892
Loughnan, M. and Nossal, G. J. V. (1989) Nature (London) **340**, 76–79
Lutzker, S., Rothman, P., Pollock, R., Coffman, R. and Alt, F. W. (1988) Cell **53**, 177–184
Margolis, B., Zilberstein, A., Franks, C., Felder, S., Kremer, S., Ullrich, A., Rhee, S. G., Skorecki, K. and Schlessinger, J. (1990) Science **248**, 607–610
Martin, M. and Resch, K. (1988) Trends Pharmacol. Sci. **9**, 171–177
McGarvie, G. M. and Cushley, W. (1989a) Cell. Signalling **1**, 447–460
McGarvie, G. M. and Cushley, W. (1989b) Immunol. Lett. **22**, 221–226
McMahan, C. J., Slack, J. L., Mosley, B., Cosman, D., Lupton, S. D., Brunton, L. L., Grubin, C. E., Wignall, J. M., Jenkins, N. A., Brannan, C. I., Copeland, N. G., Huebner, K., Croce, C. M., Cannizzarro, L. A., Benjamin, D., Dower, S. K., Spriggs, S. K. and Sims, J. E. (1991) EMBO J. **10**, 2821–2832
Melamed, I., Wang, G. and Roifman, C. M. (1992) J. Immunol. **149**, 169–174
Mercolino, T. J., Arnold, L. W. and Haughton, G. (1986) J. Exp. Med. **163**, 155–165
Merida, I. and Gaulton, G. N. (1990) J. Biol. Chem. **265**, 5690–5694
Merida, I., Pratt, J. C. and Gaulton, G. N. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 9421–9425
Metcalf, D., Nicola, N. A., Gearing, D. P. and Gough, N. M. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 4670–4674
Mills, G. B., Zhang, N., Schmandt, R., Fung, M., Greene, W., Mellors, A. and Hogg, D. (1991) Biochem. Soc. Trans. **19**, 277–287
Miyajima, A., Kitamura, T., Harada, N., Yokota, T. and Arai, K. (1992) Annu. Rev. Immunol. **10**, 295–331
Mizel, S. B. (1990) Immunol. Today **11**, 390–391
Mizuguchi, J., Beaven, M. A., Ohara, J. and Paul, W. E. (1986) J. Immunol. **137**, 2215–2220
Monroe, J. G. and Cambier, J. C. (1983) J. Exp. Med. **157**, 2073–2086
Monroe, J. G. and Haldar, S. (1989) Biochim. Biophys. Acta **1013**, 372–378
Mosley, B., Beckmann, M. P., March, C. J., Idzerda, R. L., Gimpel, S. D., VandenBos, T., Friend, D., Alpert, A., Anderson, D., Jackson, J., Wignall, J. M., Smith, C., Gallis, B., Sims, J. E., Urdal, D., Widmer, M. B., Cosman, D. and Park, L. S. (1989) Cell **59**, 335–348
Murata, Y., Yamaguchi, N., Hitoshi, Y., Tominaga, A. and Takatsu, K. (1990) Biochem. Biophys. Res. Commun. **173**, 1102–1108
Murphy, P. M. and Tiffany H. L. (1991) Science **253**, 1280–1283
Mustelin, T., Coggeshall, K. M. and Altman, A. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 6302–6306
Mustelin, T., Coggeshall, K. M., Isakov, N. and Altman, A. (1990) Science **247**, 1584–1587
Namen, A. E., Lupton, S., Hjerrild, K., Wignall, J., Mochizuki, D. Y., Schmierer, A., Mosley, B., March, C. J., Urdal, D., Gillis, S., Cosman, D. and Goodwin, R. G. (1988) Nature (London) **333**, 571–573
Nemazee, D. A. and Bürki, K. (1989) Nature (London) **337**, 562–566
Noelle, R. J. and Snow, E. C. (1990) Immunol. Today **11**, 361–368
Noelle, R. J., Krammer, P. H., Ohara, J., Uhr, J. W. and Vitetta, E. S. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 6149–6153
Noelle, R. J., Daum, J., Bartlett, W. C., McCann, J. and Shepherd, D. M. (1991) J. Immunol. **146**, 1118–1124
O'Garra, A., Warren, D. J., Holman, M., Popham, A. M., Sanderson, C. J. and Klaus, G. G. B. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 5228–5232
O'Garra, A., Rigley, K. P., Holman, M., McLaughlin, J. B. and Klaus, G. G.B (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 6254–6258
O'Neill, L. A. J., Bird, T. A. and Saklatvala, J. (1990) Immunol. Today **11**, 392–394
Oppenheim, J. J., Zachariae, C. O. C., Mukaida, N. and Matsushima, K. (1991) Annu. Rev. Immunol. **9**, 617–648
Ostergaard, H. L., Shackelford, D. A., Hurley, T. R., Johnson, P., Hyman, R., Sefton, B. M. and Trowbridge, I. M. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 8959–8963
Pardi, R., Bender, J. R., Dettori, C., Giannazza, E. and Engleman, E. G. (1989) J. Immunol. **143**, 3157–3166
Park, D. J., Rho, H. W. and Rhee, S. G. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 5453–5456
Parkhouse, R. M. E. (1990) Immunology **69**, 289–302
Paul, W. E. and Ohara, J. (1987) Annu. Rev. Immunol. **5**, 429–459
Phillips, R. J., Harnett, M. M. and Klaus, G. G. B. (1991) Int. Immunol. **3**, 617–621
Pike, B. L. and Nossal, G. J. V. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 3395–3399
Rabin, E. M., Mond, J. J., Ohara, J. and Paul, W. E. (1986) J. Immunol. **137**, 1573–1576
Rall, T. and Harris, B. A. (1987) FEBS Lett. **224**, 365–371
Ravetch, J. V. and Kinet, J.-P. (1991) Annu. Rev. Immunol. **9**, 457–492
Remillard, B., Petrillo, R., Maslinski, W., Tsudo, M., Strom, T. B., Cantley, L. and Varticovski, L. (1991) J. Biol. Chem. **266**, 14167–14170
Rigley, K. P. and Harnett, M. M. (1990) in Cytokines and B Lymphocytes (Callard, R. E., ed.), pp. 39–63, Academic Press, London
Rigley, K. P., Harnett, M. M. and Klaus, G. G. B. (1989) Eur. J. Immunol. **19**, 481–485
Rigley, K. P., Thurston, S. M. and Callard, R. E. (1991) Int. Immunol. **3**, 197–203
Rijkers, G. T., Griffioen, A. W., Zegers, B. J. M. and Cambier, J. C. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 9766–8770
Robb, R. J., Rusk, C. M., Yodoi, J. and Greene, W. C. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 2002–5658
Roehm, N. W., Leibson, H. J., Zlotnik, A., Kappler, J., Marrack, P. and Cambier, J. C. (1984) J. Exp. Med. **160**, 679–687
Rogers, J., Early, P., Carter, C., Calame, K., Bond, M., Hood, L. and Wall, R. (1981) Cell **20**, 303–312
Roifman, C. M., Wang, G., Freedman, M. and Pan, Z. (1992) J. Immunol. **148**, 1136–1142
Rolink, A. and Melchers, F. (1991) Cell **66**, 1081–1094
Rothman, P., Lutzker, S., Cook, W., Coffman, R. and Alt, F. W. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 7704–7708
Sakaguchi, N., Kashiwamura, S., Kimoto, M., Thalmann, P. and Melchers, F. (1988) EMBO J. **7**, 3457–3464
Saltzman, E. M., White, K. and Casnellie, J. E. (1990) J. Biol. Chem. **265**, 10138–10142
Samanta, A. K., Oppenheim, J. J. and Matsushima, K. (1989) J. Exp. Med. **169**, 1185–1189
Samelson, L., Phillips, A. F., Luong, E. T. and Klausner, R. D. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 4358–4362
Sanderson, C. J., Campbell, D. H. and Young, I. G. (1988) Immunol. Rev. **102**, 29–50
Saragovi, H. and Malek, T. R. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 11–15
Satoh, T., Nakafuku, M., Miyajima, A and Kaziro, Y. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 3314–3318
Scott, D. W., Chace, J. H., Warner, G. L., O'Garra, A., Klaus, G. G. B. and Quill, H. (1987) Immunol. Rev. **99**, 153–172
Secrist, J. P., Karnitz, L. and Abraham, R. T. (1991) J. Biol. Chem. **266**, 12135–12139
Seyfert, V. L., McMahon, S. B., Glenn, W. D., Yellen, A. J., Sukhatme, V. P., Cao, X. and Monroe, J. G. (1990) Science **250**, 797–800
Sharon, M., Gnarra, J. R. and Leonard, W. J. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 4869–4873
Sherman, P. A., Basta, P. V., Heguy, A., Wloch, M. K., Roeder, R. G. and Ting, J. P.-Y. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 6739–6743
Sidman, C. L. and Unanue, E. R. (1976) J. Exp. Med. **144**, 882–888
Sims, J. E., Acres, R. B., Grubin, C. E., McMahan, C. J., Wignall, J. M., March, C. J. and Dower, S. K. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 8946–8950
Smith, K. A. (1989) Annu. Rev. Cell Biol. **5**, 397–425
Smrcka, A. V., Hepler, J. R., Brown, K. O. and Sternweis, P. C. (1991) Science **251**, 804–807
Snow, E. C., Feldbush, T. L. and Oaks, J. A. (1980) J. Immunol. **124**, 739–744
Snow, E. C., Noelle, R. J., Uhr, J. W. and Vitetta, E. S. (1983) J. Immunol. **130**, 614–618
Sorensen, P., Mui, A. L.-F. and Krystal, G. (1989) J. Biol. Chem. **263**, 19203–19209
Subbarao, B., Morris, J. and Baluyut, A. R. (1988) Cell. Immunol. **112**, 329–342
Sullivan, K. A., Tyler Miller, R., Masters, S. B., Beiderman, B., Heidaman, W. and Bourne, H. R. (1987) Nature (London) **330**, 758–760
Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T. and Kishimoto, T. (1989) Cell **58**, 573–581
Takeshita, T., Goto, Y., Tada, K., Nagata, K., Asao, H. and Sugamura, K. (1989) J. Exp. Med. **169**, 1323–1332
Tavernier, J., Devos, R., Cornelis, S., Tuypens, T., Van der Heyden, T., Fiers, W. and Plaetinck, G. (1991) Cell **66**, 1175–1184
Taylor, S. J., Chae, H. Z., Rhee, S. G. and Exton, J. H. (1991) Nature (London) **350**, 516–518
Telfer, J. C. and Rudd, C. E. (1991) Science **237**, 439–441
Teshigawara, K., Wang, H. M., Kato, K. and Smith, K. A. (1987) J. Exp. Med. **165**, 223–238
Thomas, K. M., Taylor, L. and Navarro, J. M. (1991) J. Biol. Chem. **266**, 14839–14841
Tigges, M. A., Casey, L. and Koshland, M. E. (1989) Science **243**, 781–786
Tomizawa, K., Ishizaka, A., Kojima, K., Nakanishi, M., Sakiyama, Y. and Matsumoto, S. (1991) Clin. Exp. Immunol. **83**, 492–496
Tonegawa, S. (1983) Nature (London) **302**, 575–581
Tonks, N. K., Charbonneau, H., Diltz, C. D., Fischer, E. H. and Walsh, K. A. (1988) Biochemistry **27**, 8695–8701
Torigoe, T., Saragovi, H. U. and Reed, J. C. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 2674–2678
Tsokos, G. C., Lambris, J. D., Finkelman, F. D., Anastassiou, E. D. and June, C. H. (1990) J. Immunol. **144**, 1640–1645
Tsudo, M., Kozak, R. W., Goldman, C. and Waldmann, T. A. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 9694–9698
Tsujimoto, Y., Cossman, J., Jaffe, E. and Croce, C. (1985) Science **228**, 1440–1443
Turner, B., Rapp, U., App, H., Greene, M., Dobashi, K. and Reed, J. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 1227–1231
Tutt, A., Stevenson, G. T. and Glennie, M. J. (1991) J. Immunol. **147**, 60–69
Uckun, F. M., Jaszcz, W., Ambrus, J. L., Fauci, A. S., Gajl-Peczalska, K., Song, C. W., Wick, M. R., Myers, D. E., Waddick, K. and Ledbetter, J. A. (1988) Blood **71**, 13–29

Uckun, F. M., Tuel-Ahlgren, L., Obuz, V., Smith, R., Dibirdik, I., Honson, M., Langlie, M.-C. and Ledbetter, J. A. (1991a) Proc. Natl. Acad. Sci. U.S.A. **88**, 6323–6327
Uckun, F. M., Dibirdik, I., Smith, R., Tuel-Ahlgren, L., Langlie, C.-M., Schieven, G. L., Waddick, K. G., Hanson, M. and Ledbetter, J. A. (1991b) Proc. Natl. Acad. Sci. U.S.A. **88**, 3589–3593
Uckun, F. M., Schieven, G. L., Dibirdik, I., Chandan-Langlie, M., Tuel-Ahlgren, L. and Ledbetter, J. A. (1991c) J. Biol. Chem. **266**, 17478–17485
Ullman, K., Northrop, J. P., Verweij, C. L. and Crabtree, G. R. (1990) Annu. Rev. Immunol. **8**, 421–452
Van Snick, J. (1990) Annu. Rev. Immunol. **8**, 253–278
Veillette, A., Bookman, M. A., Horak, E. M. and Bolen, J. B. (1988) Cell **55**, 301–308
Venkitaraman, A. R., Williams, G. T., Dariavach, P. and Neuberger, M. S. (1991) Nature (London) **352**, 777–781
Weiss, A., Koretzky, G., Schatzman, R. C. and Kadlecek, T. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 5484–5488
Whetton, A. D., Monk, P. N., Consalvey, S. D., Huang, S. J., Dexter, T. M. and Downes, C. P. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 3284–3288
Wickremasinghe, R. G., Mire-Sluis, A. R. and Hoffbrand, A. V. (1987) FEBS Lett. **220**, 52–56
Wienands, J., Hombach, J., Radbruch, A., Riesterer, C. and Reth, M. (1991) EMBO J. **9**, 449–455
Williams, A. F. and Barclay, A. N. (1988) Annu. Rev. Immunol. **6**, 381–405
Wong, G. G. and Clark, S. C. (1988) Immunol. Today **9**, 137–139
Yamanishi, Y., Kakiuchi, T., Mizuguchi, J., Yamamoto, T. and Toyoshima, K. (1991) Science **251**, 192–194
Yanaga, F., Abe, M., Koga, T. and Hirata, M. (1992) J. Biol. Chem. **267**, 5114–5121
Yang, L., Baffy, G., Rhee, S. G., Manning, D., Hansen, C. A. and Williamson, J. R. (1991) J. Biol. Chem. **266**, 22451–22458
Zola, H., Weedon, H., Thompson, G. R., Fung, M. C., Ingley, E. and Hapel, A. J. (1991) Immunology **72**, 167–173