MINIREVIEW

Cytokines Involved in B-Cell Differentiation and Their Sites of Action (44119)

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Abstract. **B cells originate from pluripotent hematopoietic stem cells and differentiate in the bone marrow into mature B cells. The differentiation of a stem cell into a mature B cell can be subdivided into five steps: early pro-B cells, late pro-B cell stage, pre-B cell stage, immature B cells, and mature B cells. Each differentiation step appears to be regulated by co-receptor and cytokines. The earliest B-cell progenitors are bound to the stromal cell surface by adhesive interactions through cell surface molecules to promote the binding of *c-kit* to stem cell factor (SCF). At the late pro-B cell stage, interleukin-7 (IL-7) induces proliferation and differentiation of pro-B cells to pre-B cells. Surface Ig-expressing mature B cells leave bone marrow and circulate into peripheral lymphoid organs in which they can be activated to proliferate and to differentiate into antibody-secreting cells by encountering antigens and "helper" T (T_H) cells. T_H cells activate B cells by their products, cytokines such as IL-4, IL-5, and IL-6, and membrane-bound stimulatory molecules including CD40 ligand. Each cytokine has pleiotropic activity on B cells and other cell types, and acts through a specific receptor. Abnormal expression of a cytokine receptor and aberrant signal transduction causes functional abnormality of B cells.** [P.S.E.B.M. 1997, Vol 215]

B cells originate from pluripotent hematopoietic stem cells and differentiate in the fetal liver and, in adult life, principally in the bone marrow into mature B cells. Each B cell is genetically committed to become an antibody (immunoglobulin [Ig])-secreting cell. The differentiation of a bone marrow stem cell into a mature B cell can be subdivided into at least five steps—namely, early pro-B cells, late pro-B cell stage, pre-B cell stage, immature B cells, and mature B cells. Each step of B-cell differentiation is regulated by co-receptor on B cells and cytokines. The earliest B-cell progenitors are bound to the stromal cell surface through cell surface molecules whose recognition facilitate the binding of c-*kit* to stem cell factor. At the late pro-B cell stage, interleukin-7 (IL-7) induces survival and proliferation of B cells at the pre-B cell stage, and they become detached from the bone marrow stromal cell.

Surface Ig-expressing mature B cells leave the bone marrow and circulate into peripheral lymphoid organs in which they can be activated to proliferate and to differentiate into antibody-secreting cells by encountering antigens and "helper" T (T_H) cells and their products, cytokines. The cytokines (also known as lymphokines) are a set of small proteins. Subsets of these molecules are strongly related to one another genetically, structurally, and functionally. Each cytokine has multiple functions or is pleiotropic, and more than one cytokine will often be capable of mediating the same or a related function—that is, they display redundancy.

B-cell responses to antigens can be classified into two types with respect to mechanism: T cell dependent and T cell independent. Efficient B-cell responses to many protein antigens need an intimate interaction of B cells and T_H cells. The activated T_H cells provide help to B cells in two forms: soluble mediators (cytokines) such as IL-4, IL-5, and IL-6,

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and membrane-bound stimulatory molecules including CD40 ligand.

Within the last 10 years, enormous information has accumulated about cell surface functional molecules and cytokines involved in B-cell development and triggering. In this review, I will discuss cytokines involved in B-cell development and differentiation, as well as their sites of action.

Cytokines Involved in Early B-Cell Development

Development of B cells from their progenitor cells has been extensively studied. The differentiation of a bone marrow stem cell into a mature B cell can be subdivided into at least five steps (1–5). An early step results in the production of early pro-B cells, which appear before Ig gene rearrangement has begun, and are identified by other surface markers characteristic of B cells. The subsequent stages are defined by steps in the rearrangement of Ig genes and by changes in cell surface markers, dependence on growth factors, and location in the bone marrow. At the second step (the late pro-B cell stage), D_H-J_H joining has occurred, and at the third step (the pre-B cell stage) intact heavy chains are produced (6). At the fourth step, the light-chain genes undergo rearrangement, leading to the expression of a complete Ig on the cell surface to produce immature B cells. Finally, immature B cells differentiate within a few days into mature B cells expressing surface IgM and IgD (the fifth step).

The earliest B-cell progenitors are bound to the stromal cell surface by adhesive interactions through cell surface molecules, including VCAM-1 on the stromal cell and LFA-1 on the B-cell progenitor (7–9). Such recognition may facilitate the binding of a receptor known as c-*kit* on the B-cell precursor to a second stromal cell surface molecule, known as stem cell factor (SCF). Tyrosine kinase activity of c-*kit* is activated by binding of SCF resulting in stimulation for proliferation of the early pro-B cell (10). At the late pro-B cell stage, receptors for IL-7 appear on the cell surface. IL-7 is a cytokine secreted by bone marrow stromal cells, and it induces survival and proliferation at the pre-B cell stage (11), when the developing B cells lose their dependence on SCF, cease to express c-*kit* and become detached from the bone marrow stromal cell (12, 13). Most models of hematopoiesis suggest that lymphocyte progenitors differentiate from common stem cells very early in maturation, so that IL-7 is probably acting on cells at the same levels of development as IL-3 or GM-CSF. Recent studies suggest that IL-7 may also stimulate the growth and maturation of immature $CD4^-CD8^-$ T cell progenitors in the thymus (14). However, this is based on *in vitro* experiments, and the cellular source of IL-7 in the thymus is not known. Transgenic mice that overexpress IL-7 show markedly increased numbers of pre-B cells in the bone marrow and peripheral lymphoid tissues.

Functional Molecules Involved in B-Cell Activation

B cells bind antigenic determinants on soluble proteins or those on bacteria utilizing cell surface Ig receptors. That interaction by itself does not stimulate the B cell but initiates the process of endocytosis, and some of the antigen, bound to receptor, is brought into the cell where it is proteolyzed. Some of the resulting peptides are bound by MHC class II molecules in the cytosol and transported to the cell surface as a complex, where T_H cells with receptors specific for that peptide/MHC complex recognize them and become activated. As a result of this activation, the T_H cell produces new molecules, both expressed on its cell surface and secreted, which activate B cells and cause the B cells to develop into antibody-producing cells, as well as mediating other important changes.

B-Cell Activation by Cognate T_H Cell Help. Optimal activation of B cells by T_H cells requires direct contact between the two cell types (15–18). Helper activity can be provided by either fixed, activated T cells (17) or by purified membrane preparations from activated T cells (19–21). These findings suggest that activation of T_H cells results in the expression of a cell surface molecule that can stimulate B cells. The identification of CD40 ligand (CD40L) (gp39) expressed by activated T_H cells was important for elucidating the molecular mechanism through which cognate T_H cell help is provided to B cells. CD40L is a 33-kDa type II membrane glycoprotein with sequence homology to tumor necrosis factor (22, 23). T cells activated through TCR ligation transiently express on their surface CD40L, which interacts with CD40 expressed on B cells to deliver signals for B-cell activation and proliferation. Recombinant murine CD40L directly induces murine B-cell proliferation and expression of elevated levels of cell surface class II MHC and CD23 molecules (23). This effect is positively regulated by other cytokines, most notably IL-4 and IL-5. By itself, CD40L has no effect upon Ig secretion by B cells. However, when B cells are treated with CD40L plus cytokines, Ig secretion is stimulated in a cytokine-dependent and isotype-specific manner. IL-4 is a potent co-stimulator of IgE and IgG1 in the presence of CD40L, and IL-5 acts synergistically with IL-4 in these responses as well as in IgM and IgG3 production (24). These findings indicate that CD40L plays a critical role in signaling during cognate T-cell/B-cell interactions.

CD40 is a 48-kDa membrane glycoprotein expressed on B cells during various stages of development (25, 26). Engagement of CD40 on human B cells *via* CD40 mAb triggers a variety of functions, including homotypic adhesion (27, 28), increased cell size (28, 29), short- (25, 28–30) and long-term (31) proliferation, and rescue from apoptosis (32). CD40 mAb in combination with IL-4 stimulates Ig secretion, including a dramatic increase in IgE levels in the presence of IL-4 (33–35). The latter function has been further characterized on the molecular level by germ-line Cε

transcription and switch recombination (36). CD40 expressed on B cells serves as a receptor for T-cell surface CD40 ligand. In this regard, antagonists of the interaction of CD40 with its ligand inhibit the T cell–dependent activation of B cells in both murine and human systems (37, 38).

The CD40-CD40L interaction is also important for other B-cell functions, including the formation of germinal center and the induction of memory B cells. The physiological significance of the CD40-CD40L interaction *in vivo* has become evident from the observation of patients with X-linked hyper-IgM (XHM) syndrome, who have mutations in the gene encoding CD40L (39–41) that cause alterations in the CD40L protein rendering it incapable of binding to CD40. As a result, helper T cells in the patients cannot trigger B-cell activation and Ig production. This disease is characterized by reduced levels of serum IgA, IgG, and IgE. The patients fail to produce Ig of isotypes other than IgM in response to antigen challenge. These patients have a complete absence of germinal centers in their secondary lymphoid organs. Over the past several years, insights into the function of CD40-CD40L in the regulation of humoral immune response have been provided by studies in CD40L-deficient (42, 43) and CD40-deficient (44, 45) mice, and in mice treated with anti-CD40L (46, 47) or a soluble form of CD40 (48). For the most part, all of these systems agree that CD40L and CD40 interactions are essential for secondary responses to T-dependent antigens and in the formation of germinal centers.

The cognate interaction of B and T cells triggers the engagement of many other accessory molecules on both cells, which serve to stabilize the B-T conjugation and to coordinate the responses of the interacting cells. The accessory molecules are B7-1 (CD80), B7-2 (CD86), CTLA-4, LFA-1 (CD11a/18), LFA-2 (CD2), LFA-3 (CD58), ICAM-1 (CD54), VCAM (CD106), and VLA-4 (CD49d/29) (49, 50). The B7-1/B7-2 expressed on B cells interacts with the CD28/CTLA-4 expressed on T cells to deliver a co-stimulatory signal to TCR-stimulated T cells (51, 52), which then become fully activated to produce cytokines. T cell–derived cytokines such as IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and γ-interferon (IFN-γ) have been shown to enhance B-cell activation and proliferation, and to be essential co-stimuli for subsequent B-cell differentiation and Ig production.

B-Cell Activation through the BCR. The B-cell receptor (BCR) is a complex with hetero-oligomeric structure in which antigen recognition and signal transduction are compartmentalized into distinct subunits (1, 2, 53). The antigen recognition subunit of the BCR is a tetrameric complex of heavy and light Ig chains, whereas the signal transduction subunit is a disulfide-linked heterodimer composed of Igα (CD79a), a *mb-1* gene product, and Igβ (CD79b), a *B29* gene product. Both Igα and Igβ chains contain within their cytoplasmic domains a sequence motif (ITAM) which is also found in the cytoplasmic tails of signal transducers of T cell and Fc receptors (53).

Stimulation of B cells through the BCR results in rapid increases in tyrosine phosphorylation on a number of proteins and induces an increase of phosphatidylinositol and mobilization of cytoplasmic free calcium. Though none of the BCR subunits contains intrinsic protein tyrosine kinase (PTK) activity, the BCR associates with two types of cytoplasmic PTKs: Src family PTKs including Lyn, Fyn, Blk, and Lck, and the more distantly related PTK, Syk. Activation of these PTKs through ligation of BCR by antigen leads to the phosphorylation of two tyrosine residues within ITAM in Igα and Igβ chains of BCR, which in turn leads to recruitment and activation of additional PTKs (53). The activated PTKs include Btk and appear to phosphorylate numerous cellular proteins involved in intracellular signaling pathways, such as adapter protein Shc in the Ras pathway, phospholipase C (PLC)-γ2, GTPase-activating protein (GAP), mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3-kinase), and Vav (54, 55).

The regulated signal transduction through BCR can be achieved by coordinated actions of PTKs and protein tyrosine phosphatases (PTPs) (56, 57). CD45 is a transmembrane PTP expressed on hematopoietic cells, and it plays a critical role in B-cell activation following ligation of BCR. The regulation of Src family PTKs by CD45 appears to account in part for the requirement of CD45 in antigen-induced BCR signaling. Since Src family PTKs contain a negative regulatory tyrosine phosphorylation site at their carboxyl termini, dephosphorylation of this site by CD45 results in increased kinase activity. The phosphorylated tyrosine residues within the ITAMs of Igα and Igβ chains of BCR appear to be substrates for CD45 as well. The importance of CD45 in BCR signaling has been confirmed by generating CD45-deficient mice in which B cells are completely refractory to proliferation stimulated by anti-IgM antibody (58).

B-Cell Activation through CD38. CD38 is a 42-kDa membrane glycoprotein expressed in many cell types including B cells and has been shown to associate with the BCR in humans (59, 60). Ligation of CD38 with agonistic antibodies induces a potent proliferative signal to B cells and the rescue of B cells from apoptosis induced by irradiation and dexamethazone. Engagement of CD38 also rescues human tonsillar B cells in the germinal center from apoptosis. In the murine system, CD38 ligation and IL-5 synergistically stimulate resting B cells for maximum proliferative responses. CD38 ligation by itself has no effect upon the expression of B lymphocyte–specific maturation protein-1 (Blimp-1) and Ig-secretion by B cells. However, when B cells are treated with anti-CD38 mAb plus IL-5, Blimp-1 expression and Ig secretion are induced in an IL-5–dependent and isotype-specific manner (61). CD38 stimulation activates Btk tyrosine kinase and augments the expression of the IL-5Rα chain (61). Murine X-linked immunodeficiency (Xid) results in a failure of B cells to become phenotypically and functionally diverse. Xid mice have defects manifested by a decrease in the overall number

of B cells, low levels of circulating IgM and IgG3, a high surface IgM-to-IgD ratio, and failure in responding to type II thymus-independent antigens, or CD40 ligation. Recently, Xid mice have been shown to carry a mutation in the *btk* gene (62, 63). We found that CD38 ligation did not induce proliferation of B cells from Xid mice (61).

Cytokines Involved in B-Cell Differentiation

After the discovery of T_H-B cell collaboration in the antibody response, it was originally considered that T_H cells represent a single subset in the T cell lineage. However, several investigators recognized that antigen-specific B-cell responses can be triggered in certain circumstances where B and T_H cells are independently stimulated by determinants present on two distinctly separate molecules. The mechanism underlying such B-cell triggering was interpreted to be the mediation of the T_H cell effect *via* the release of cytokines (64–67). Evidence for the existence of such mediators came initially from the demonstration that supernatants obtained from short-term cultures of histoincompatible mouse spleen cells contained a nonspecific biologically active mediator capable of markedly affecting *in vitro* antibody responses to T-dependent antigens. One of these cytokines was originally designated as T cell–replacing factor (TRF) because it induced terminal differentiation of late-developing B cells to Ig-secreting cells (68–70). Since this observation, a number of factors relevant to T_H-B cell interactions have been described by other investigators. Biochemical characterization of TRF took a long time, in part because of the popular belief that the different activities should be ascribed to separate biochemical entities and partly because purification of TRF was difficult.

In the early 1980s, it was reported that T_H cell–dependent B-cell activation involves both a proliferative phase and a separate state of differentiation (71, 72). Furthermore, it was shown that at least two different kinds of cytokines were required in the regulation of B-cell responses, one for growth of activated B cells, (B-cell growth factor [BCGF]), and the other for antibody induction of B cells, (B-cell differentiation factor [BCDF]). Moreover, investigators realized that the source of TRF contains various factors, including T-cell growth factor (IL-2) and lymphocyte-activating factor (IL-1), both of which can induce B-cell growth and differentiation. A series of experimental approaches were then used to characterize the nature of TRF (then called B cell–stimulatory factor [BSF]) that can induce B-cell growth and differentiation, and to clarify the precise roles of BSFs that induce B-cell development. However, this was hampered by the pluripotency of some BSFs, by the synergistic and inhibitory interactions among BSFs, and by the complexity of various BSF bioassays.

BSF-1, IL-2, and BCGFII were all claimed to act as BCGF. A number of factors were also found to act as BCDF. These include TRF, BCGFII, BSF-2, and IL-2. The cDNAs for three distinct BSFs were then cloned: (i) IL-4 (73, 74), which causes B-cell activation, growth, and differentiation; (ii) IL-5 (75, 76), which induces B-cell growth and differentiation with diverse activities; and (iii) IL-6 (77), which induces differentiation of B cells without inducing cell proliferation. Studies of IL-4 revealed that a single cytokine exerts a variety of activities on diverse target cells (78). Together with similar properties of other recombinant cytokines, these results helped immunologists escape the beliefs that one cytokine is required for each activity and that growth factor activity and differentiation factor activity are necessarily different molecular entities. All recombinant cytokines that act on B cells are also active on inflammatory cells whose growth and differentiation can be involved in inflammation (78–80).

Role of IL-4 in B-Cell Activation. IL-4 is a T cell–derived glycoprotein first described as a growth factor for highly purified small B cells stimulated with suboptimal doses of anti-IgM antibodies. The factor was initially designated BCGF and was subsequently renamed BCGFI and then BSF-1 (81). The cDNA encoding BSF-1 was cloned, and studies using recombinant BSF-1 revealed that it has pleiotropic activity on various target cells (73, 74). BSF-1 was finally designated IL-4, and the IL-4 cDNA encodes 140 amino acids with a signal sequence of 20 residues. The secreted core polypeptide contains 120 residues with a molecular mass of 14,137 (74). Human IL-4 shows 50% DNA sequence homology with murine IL-4 at the level of amino acid sequence. The principal cellular sources of IL-4 are $CD4^+$ T_H, cells specifically of the T_H2 subset. In fact, IL-4 production is used as the criterion for clarifying $CD4^+$ T cells into this subset (see below), with IFN-γ being the hallmark of the T_H1 cells. Activated mast cells and basophils, as well as some $CD8^+$ T cells, are also capable of producing IL-4.

Initially, IL-4 was thought to be a "growth factor" that acts early in G1 phase to initiate DNA synthesis. However, experimental results indicated that IL-4 acts on resting (G0 phase) B cells in the absence of any co-stimulant: (i) IL-4 induces an 8- to 10-fold increase in class II MHC antigen expression on resting B cells, (ii) IL-4 causes a small but significant increase in cell volume of resting B cells, and (iii) pretreatment of resting B cells with IL-4 alone for a period of 24 hr promoted subsequent entry of cells into S phase and their subsequent response to anti-IgM and IL-4 by about 12 hr. All of these results indicate that IL-4 is multifunctional and exerts its action on resting B cells. It is not a simple growth factor but, rather, an "activation factor."

B-cell differentiation–inducing activity. IL-4 also functions as a differentiation factor of B cells in certain situations. In general, murine B cells treated with LPS will secrete IgM and IgG3, but little or no IgG1. It was shown, however, that the addition of IL-4 results in the secretion of IgG1 and the partial suppression of IgG3 and IgG2b production. It has little to no effect on IgM production. In this

capacity, IL-4 was originally designated B-cell differentiation factor for IgG1 (BCDFγ) (18), or IgG1 induction factor (82). IL-4 shows an increase in the levels of steady-state γ1-mRNA expression with a concomitant decrease in γ2b- and γ3-mRNA levels in surface IgG1-negative B cells, indicating that IL-4 is an isotype "switch" factor. IgE is the principal mediator of immediate hypersensitivity (allergic) reactions, and enhanced production of allergies. IgE antibodies also play a role in defense against helminthic infections, this being the principal known physiologic function of the T_H2 subset of T_H cells. Mice in which the IL-4 gene is disrupted fail to produce IgE. IL-4 also inhibits switching to IgG2a and IgG3 in mice, switching that is augmented by IFN-γ and is thus one of several reciprocal actions of IL-4 and IFN-γ.

Intriguingly, IL-4 was also demonstrated to induce the expression of FcεRII on B cells. Since FcεRII is believed to be involved in the regulation of IgE synthesis, IL-4 may be involved in the regulation of immediate-type hypersensitivity in two different ways: one as BCDF for IgE secretion and the other as an inducer of FcεRII receptor expression. IL-4 also has activities named as mast cell growth factor–2 (MCGF-2) and T-cell growth factor–2 (TCGF-2) (73), distinct from IL-2. It is now known that the main physiologic function of IL-4 is as a regulator of allergic reactions. IL-4 is a member of the four α-helical cytokine family.

T-cell development and subsets. Because of the importance of cytokine-producing pattern of T cells, it is a matter of both scientific and practical interest to understand the relative regulation of the production of IL-4 and IFN-γ as well as control of the appearance of T cells that can make these molecules. Stimulation of naïve resting T cells with antigens or stimulants that mimic antigen causes these cells to produce IL-2. However, very few of these cells (less than one per thousand) produce IL-4 upon their first encounter with antigen. Long-term lines of antigen-specific $CD4^+$ T cells appear to be of two major types, those that secrete IFN-γ and IL-2, and those that produce IL-4, IL-5, IL-6, and IL-10 upon stimulation. The former have been designated T_H1, and the latter T_H2 cells (83). Paul and his associates used polyclonal stimulants such as immobilized antibodies to a component of the TCR to determine that IL-4 itself had a remarkable role in determining the appearance of IL-4–producing cells (84). The addition of IL-4 to the priming culture resulted in excellent preparation for production of IL-4 upon rechallenge and profoundly diminished production of IFN-γ upon restimulation. Cells primed in the presence of IL-4 were also inhibited in their capacity to produce IL-2 upon restimulation.

The means through which IL-4 determines the pattern of cytokines produced in primed cells is an issue of considerable interest. Small resting $CD4^+$ T cells from naïve donors stimulated with anti-CD3 and a source of antigen-presenting cells (APC) produce substantial amounts of IL-4 within 2 days. However, if the cells are treated with IL-4 during this period, IL-2 and IFN-γ production is diminished by more than 30-fold. This effect depends on the presence of IL-4 during the process of T-cell activation. Pre-culture of T cells with IL-4 without anti-CD3 or without APC does not diminish subsequent production of IL-4, nor does preculture of the antigen-presenting cells in IL-4 have any effect. These results demonstrate that IL-4 has striking ability to regulate the pattern of cytokine production.

IL-4 signal transduction. IL-4 exerts its effect by interacting with specific cell-surface receptors (IL-4R) and initiating a cascade of intracellular events which lead to cellular response. The IL-4R has been shown to be present on both hematopoietic and non-hematopoietic cells (85, 86). A single class of high-affinity binding sites have been detected on a wide variety of cells, including B and T cells. cDNAs encoding both murine and human IL-4R have been cloned (87, 88). The IL-4 binding protein is a type I membrane protein consisting of an extracellular domain, a single transmembrane domain, and a relatively long cytoplasmic tail. The extracellular domain contains motifs conserved among the cytokine receptor superfamily: four cysteine residues at a fixed distance and a Try-Ser-X-Try-Ser (WSXWS) motif (89). The intracellular domain of IL-4R does not have a consensus sequence for protein kinases and phosphatases. Upon transfection of cDNA for IL-4R, a single 140-kDa protein was expressed in COS cells. Soon after that finding, it was determined that the transfection of IL-4R cDNA into IL-3–dependent cell lines failed to transduce IL-4 signals for proliferation, raising the possibility that another protein may be involved in constructing the functional IL-4R.

The high-affinity IL-2R is composed of three distinct molecules, namely α-, β-, and γ-chain (90, 91). Each molecular component of the IL-2R was molecularly cloned (90–92), and functional analysis of cells expressing each component revealed that not only IL-2Rβ but also IL-2Rγ is essential for intracellular signal transduction (92). Expression of IL-2Rγ is detectable in a wide range of hematopoietic cell populations as distinct from IL-2Rα and IL-2Rβ expression. Intriguingly, IL-2Rγ was shown to be signal transducer of IL-4 together with IL-4–binding protein (IL-4Rα) by reconstitution experiments of the functional IL-4R and by blocking of IL-4 function with the use of anti–IL-2Rγ mAb (93, 94). Human X-linked severe combined immunodeficiency disease (XSCID) is characterized by severe impairment of humoral and cell-mediated immunity, which can be cured by successful bone marrow transplantation (95). All the patients with XSCID have mutations for the IL-2Rγ gene (91, 95) resulting in complete or profound deficiency of T cells. As the IL-2Rγ chain is a signal transducer for IL-4R, the mutated IL-2Rγ in XSCID patients may impair the development of T cells in response to IL-4.

IL-4 stimulation induces rapid tyrosine phosphorylation and activation of non-receptor tyrosine kinase JAK1 and JAK3 resulting in tyrosine phosphorylation and activation of signal transducers and activators of transcription 6 (STAT6) (96, 97). The importance of activation of STAT6 in IL-4 signaling was further ascertained by using STAT6-

deficient mice who had impaired IL-4–specific signal transduction (98, 99).

IL-5 and B-Cell Growth and Differentiation. The establishment of a TRF-producing T-cell hybrid, B151K12 (100), which does not secrete detectable levels of other lymphokines affecting B cells, and the production of anti-TRF mAb (101) demonstrated that TRF is a novel lymphokine. TRF was subsequently shown to promote growth of dextran sulfate-stimulated normal B cells or a murine leukemic B-cell line BCL1 (102, 103), an activity also known as BCGF II. Molecular cloning of cDNAs encoding murine and human TRF convincingly demonstrated that a single molecule is responsible for TRF and BCGFII activities (76). Although TRF was initially believed to be principally active on B cells, recombinant TRF has been shown to have wide variety of activities on various target cells (104, 105). For that reason TRF was designated IL-5.

IL-5 is a glycoprotein induced in T_H2 cells after stimulation with antigen such as *Mycobacterium tuberculosis* (106) or *Toxocara canis* (107), and in mast cells upon stimulation with allergen/IgE complex or calcium ionophore (108). IL-5 is an interdigitating homodimeric glycoprotein and a member of the four α-helical bundle motif, which is conserved among several hematopoietic cytokines. While initially identified by its ability to support the growth and differentiation of B cells, IL-5 is now known to have pleiotropic effects on the immune system and inflammation (109). Two mAbs, NC17 and TRFK4, have been widely used because of their ability to neutralize IL-5 function both *in vitro* and *in vivo* (110–112). A number of IL-5–dependent mouse B-cell lines have now been isolated and have provided a convenient biological assay for IL-5 (113). IL-5 induces differentiation of activated conventional B (B-2) cells into Ig-secreting cells and induces the growth of progenitors of $CD5^+$B (B-1) cells and IgM production by B-1 cells.

In humans, the biologic effects of IL-5 are best characterized for eosinophils. In addition to inducing terminal maturation of eosinophils (68) and eosinophilic bronchopulmonary inflammation of asthma, IL-5 prolongs eosinophil survival by delaying apoptotic death, possesses eosinophil chemotactic activity, increases eosinophil adhesion to endothelial cells, and enhances eosinophil effector function (114–117). In several pathophysiological conditions, an increase in serum and tissue levels of IL-5 and eosinophil numbers have been described. These diverse biological consequences of IL-5 provided impetus for elucidating the functional structure of IL-5 and its receptor complex, as well as the mechanisms of IL-5 signal transduction.

Regulation of Ig production. The effect of IL-5 on Ig production has been investigated in detail in the mouse (109). Murine IL-5 induces polyclonal IgM, IgG1, and IgA production through different mechanisms. IL-5 stimulates polyclonal IgM production by the BCL_1 line and *in vivo*–activated B-cell blasts by inducing an increase in the level of mRNA for the secreted forms of IgM.

IL-5 acts on surface IgA-positive ($sIgA^+$) B cells, but not on $sIgA^-$ B cells, to induce antigen-specific and polyclonal IgA production by antigen-primed B cells and lipopolysaccharide (LPS)-stimulated B cells, respectively. Thus IL-5 appears to act on B cells committed to become IgA-secreting cells (118–122). Transforming growth factor–β1 (TGFβ) was also shown to enhance IgA production by LPS-stimulated mouse B cells (121, 122), and synergized with IL-5 to induce IgA synthesis (122). TGFβ acts on $sIgA^-$ cells to induce class-switching from μ- to α-chains determined by expression of sterile α-chain transcripts and sIgA expression. TGFβ also enhances IL-5R expression, whereas IL-5 induces B cells, in which the μ-chains have already switched to α-chains, to differentiate into IgA-secreting cells. TGFβ/LPS stimulation induces switching from μ- to α-chains as confirmed by the appearance of germ-line Cα transcripts, by the analysis of IgA-specific switch circular DNA, and by the frequency analysis of IgA-secreting cells. IL-5 induces neither germ-line Cα transcripts nor the formation of IgA-specific switch circular DNA. TGFβ enhances not only IgA but also IgG2b, and to a lessor extent IgG3, production by LPS-stimulated B cells at optimum conditions. In this case, IL-5 does not synergize with TGFβ for IgG2b or IgG3 synthesis. These results indicate that TGFβ is a class-switching factor for μ- to α-chains and μ- to γ2b-chains as well, and that IL-5 can synergize with TGFβ only for IgA synthesis.

IL-5 also promotes IgG1 and IgE secretion in the presence of IL-4. Although IL-4 plays an important role in the regulation of μ- to γ1-chain or μ- to ε-chain switching, in combination with IL-4, IL-5 induces marked accumulation of productive γ1- and ε-chain transcripts (123). These observations suggest that IL-5 may promote switch recombination, enhance transcription of rearranged γ1- and ε-chain loci, or increase the stability of the VDJ-Cγ1 and VDJ-Cε mRNA.

Promotion of B-1–cell growth and differentiation. B-1 cells have numerous noteworthy characteristics, such as their self-replenishing ability, particular tissue distribution (abundant in the peritoneal and pleural cavity), and production of autoantibodies (124, 125). B-1 cells form a minor population of the total splenic B-cell pool and are absent from lymph nodes. They thus represent a subpopulation of B cells (B-1 cells) with distinct physiologic properties from conventional B cells (B-2 cells). Moreover, the published data demonstrate failure of bone marrow cells to reconstitute B-1 cells, whereas either fetal liver (presumably precursors) or B-1 cells from the peritoneum (mature cells) can reconstitute. B-1 cells are thought to be a major contributor of IgM in the serum. Their antibody repertoire is dominated by a restricted set of V genes, and they have been considered carriers of "natural" immunity as well as autoreactive antibodies. Coupled to the observation that autoimmune mice have a higher number of B-1 cells than normal mice, this has led to the suggestion that B-1 cells play a role in the development of autoimmune diseases.

In normal mice, IL-5R expression is readily demonstrated on B-1 cells that resides largely in the peritoneum (126). The B-1 progenitors develop along a stromal cell–dependent IL-5–sensitive pathway (113), which can bifurcate to $CD5^+$ macrophages under the influence of GM-CSF (127). In mice expressing the IL-5 transgene, the B-1 cells are markedly increased with concomitant hypergammaglobulinemia and autoantibody production (128–130). Likewise, IL5–responsive B-1 cells are increased in the spontaneously autoimmune NZB and (NZB × NZW) F1 mice (131). The potential for $CD5^-$ B (B-2) cells to express the IL-5R complex and respond to IL-5 can also be demonstrated. Thus, evidence has accumulated in murine systems for both an IL-5–sensitive developmental pathway for B-1 cells and a role for IL-5 in the terminal differentiation of mature B-1 cells or appropriately activated B-2 cells. The ability of IL-5 to prevent apoptosis of B cells also suggests a potential role for IL-5 in the survival of germinal center B cells.

B-1 cells are reported to be absent in the peritoneal cavity of Xid mice. It has been reported that B cells from Xid mice respond poorly to IL-4 and IL-10, and show a decrease in the number of peritoneal IL-5Rα$^+$ B cells (132). Furthermore, the frequency of precursors of IL-5–responsive B cells in Xid mice is approximately a 100-fold lower than that of normal mice. However, Xid mice show IL-5–induced eosinophilia in peripheral blood to an extent similar to that in normal mice. We generated IL-5Rα transgenic (5Rα-Tg) mice carrying the mouse IL-5Rα gene ligated with the human IgH enhancer and mouse V_H promoter. Then, 5Rα-Tg mice carrying the *xid* gene (Xid-5Rα-Tg) were generated by crossing C57BL/6.*xid* female mice with 5Rα-Tg male mice (133). The majority of spleen B cells and B220-positive bone marrow cells from Xid-5Rα-Tg mice expressed IL-5Rα. Numbers of B-1 cells in both the Xid-5Rα-Tg and Xid mice were strikingly reduced. These results imply that the absence of B-1–cell population in Xid mice is not due to decreased expression of IL-5Rα. Xid-5Rα-Tg mice showed no significant anti–TNP-ficoll IgM antibodies, as in the case of Xid mice. These results demonstrated that the enforced expression of IL-5Rα in Xid mice had no effect on the abnormal B-cell development caused by the *xid* mutation. B cells from Xid-5Rα-Tg mice did not respond to IL-5, although all B cells expressed IL-5Rα. Thus, the low responsiveness of B cells to IL-5 in Xid mice is intrinsic to B cells, and IL-5–mediated signaling cascades in eosinophil are different from those in B cells. In conclusion, the *xid* gene product affects mIL-5 responsiveness of B cells, but not eosinophils.

As we discussed above, Xid mice have an amino acid substitution of Btk which may partially inhibit Btk function. The substitution of Arg at position 28 to Cys of Btk in Xid mice may perturb recognition of an as yet unidentified Btk-associated protein, resulting in the alteration of IL-5R–mediated signaling in B cells from both Xid and Xid-5Rα-Tg. The discovery of cellular molecule(s) that can interact with the mutated Btk, through its amino-terminal unique region, should shed light on the B cell–specific defect in Xid mice.

Rapidly emerging information on the characteristics of mice in whom the genes for IL-5 or IL-5Rα is disrupted is clarifying the role of IL-5 in the development of B cells and in B-cell function. Successful generation and breeding of mice with these genetic deficiencies indicate that none of these mutations is lethal. Disruption of either IL-5 or IL-5Rα expression results in a reduction in basal levels of eosinophils (134, 135). However, B cells in both the B-1 and B-2 lineages are normal in the adult mice, but the neonatal IL-$5^{-/-}$ as well as IL-5Rα$^{-/-}$ mice have a reduction in B-1 cells. The delayed establishment of normal members of B-1 cells in these mice suggests that IL-5 may be important but is not essential for the development of this lineage. Disruption of the IL-5Rα chain results in a reduction in serum IgM and IgG3 levels (135). The IL-$5^{-/-}$ and IL-5Rα$^{-/-}$ mice unexpectedly have morphologically normal eosinophils which are present in small numbers. Infection of IL-5Rα$^{-/-}$ mice with *Angiostrongylus cantonensis* showed delayed expulsion of worms, suggesting that IL-5–induced eosinophils play an important role in protective immunity against some types of nematode infection.

IL-5 signal transduction. IL-5 acts on target cells by binding to its specific receptor (IL-5R). The IL-5R consists of a unique α (IL-5Rα)-chain (136–139) and a β (βc)-chain that is shared with IL-3R and GM-CSFR (139–144). The βc-chain is indispensable for signal transduction. Both subunits contain motifs conserved among the superfamily of cytokine receptors. Although we and others determined that the tyrosine phosphorylation of cellular proteins is required for IL-5R–mediated signaling, the precise molecular mechanisms of IL-5R–mediated signaling are not clear. IL-5 stimulation of cells induces rapid tyrosine phosphorylation of various cellular proteins including the βc-chain and activates Btk and JAK2 kinases (145, 146). Both the cytoplasmic domain of the βc-chain and the membrane proximal proline-rich sequence of the cytoplasmic domain of IL-5Rα are essential for the IL-5–induced proliferative response (145, 146), for the expression of nuclear proto-oncogenes, and for activation of Btk and JAK2 kinases.

IL-6 and B-cell differentiation. Human IL-6 was originally identified as a factor (designated BCDF and subsequently BSF-2) in the culture supernatants of mitogen- or antigen-stimulated peripheral mononuclear cells, which induced Ig production in Epstein-Barr virus (EBV)–transformed B-cell lines or in *Staphylococcus aureus* Cowan I (SAC)–stimulated normal B cells (6, 10, 147, 148). This molecule was found to be separable from other cytokines, BSF-2 was purified to homogeneity from the culture supernatant of a human T-cell leukemia virus type I (HTLV-1)–transformed T-cell line, and its partial amino-terminal amino acid sequence was determined. Based on these findings, the cDNA for IL-6 was cloned (77). The IL-6 cDNA encodes 212 amino acids, including 28 strongly

hydrophobic amino acid residues at the N terminus which are cleaved off during processing.

At the same time, others recognized that the IL-6 sequence was identical to that published for human 26-kDa protein (149) and IFN-β2 (150). The results revealed that BSF-2, IFN-β2, and the 26-kDa, protein were identical. Growth factors for hybridoma/plasmacytomas (HPGF) have been reported by several investigators (151). In 1986, the N-terminal amino acid sequence of human HPGF was determined and found to be identical to that of BSF-2/IFN-β2/26-kDa protein (152, 153). Subsequently cDNA cloning of murine HPGF was completed, and the sequence indicated that it was the murine homolog of IL-6/BSF-2 (154, 155).

IL-6 is produced by human fibroblast after treatment with IL-1, poly(I):poly(C), and/or cycloheximide. It is also synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells in response to IL-1 and, to a lesser extent, TNF (156). It is made by some activated T cells. IL-6 can be detected in the circulation following gram-negative bacterial infection or TNF infusion and appears to be secreted in response to TNF or IL-1 rather than to LPS. IL-6 does not cause vascular thrombosis or the tissue injury that is seen in response to LPS or TNF. More recently, the abnormal production of IL-6 in autoimmune disease such as rheumatoid arthritis was considered (157). It was also reported that several tumors such as cardiac myxoma, Castleman disease, cervical cancer cells, and bladder carcinomas aberrantly produce large amounts of IL-6 (157, 158). The two best-described actions of IL-6 are on B cells and hepatocytes.

Induction of B-cell differentiation. Studies with recombinant IL-6 confirmed the predicted ability to induce terminal maturation of B cells into antibody producing cells. IL-6 augments the production of IgM, IgG, and IgA in Pokeweed mitogen (PWM)–stimulated PBL. IL-6 is also effective for increasing antibody production in mice primed with T-dependent antigens. An anti–IL-6 mAb completely abrogated PWM-induced Ig-production in PBL, whereas mitogen-induced proliferation of B cells was not affected, indicating that IL-6 is not involved in the growth of activated B cells. The influence of IL-6 is not restricted to B cells, and it can also act on resting T cells which express IL-6R (156). In fact, IL-6 induces the expression of IL-2R on T cells and IL-2 production in mitogen-stimulated T cells and thymocytes. So IL-6 may affect B-cell triggering by two different mechanisms: one through a direct effect on B cells and the other through activation of T cells.

When antibody-producing cells become cancerous, in multiple myeloma, IL-6 was found to be a potent growth factor for the tumor (159). Kishimoto and colleagues reported that myeloma cells themselves produce IL-6. Myeloma cells have IL-6 receptors, and IL-6 increase their rate of growth dramatically. If, however, anti–IL-6 antibody is used to neutralize the activity of IL-6, the rate of growth of myeloma cells can be controlled. These results imply that IL-6 is a autocrine growth factor for myeloma cells, which both produce IL-6 and use it to grow. It was of interest to know whether IL-6 in antibody-producing B cells would cause myeloma in the mice. IL-6-Tg mice had oversized spleens and lymph nodes, were diagnosed as typical cases of plasmacytoma, and died within a few months. While plasma cells were increased in number, they were not yet cancerous (160, 161). The kidneys of the IL-6-transgenic mice were found on dissection to have mesangial cell proliferative nephritis, indicating that the abnormal production of IL-6 leads to the development of the nephritis.

Induction of acute-phase protein. Interestingly, IL-6 exerts a stimulatory effect (102, 103) on hepatocytes to induce the synthesis of acute-phase proteins. Burns on the skin, pneumonia, or inflammation in some part of the body cause the liver to stop production of albumin and switch to producing a range of proteins called acute-phase proteins. These include C-reactive protein (CRP), β2-fibrinogen, amyloid protein, and various protease inhibitors. A factor that was initially called hepatocyte stimulation factor (HSF) stimulates liver cells to produce acute-phase proteins, and HSF is now known to be IL-6 (162, 163). IL-6 also interacts with hematopoietic stem cells in order to activate them. Perhaps most interesting of all is the finding that IL-6 stimulates the development of megakaryocytes, the precursors of platelets, bringing them to maturity and increasing numbers of platelets.

IL-6 receptor and signal transduction. IL-6 provides multiple signals on various tissues and cells, and the signal is transduced through IL-6R. The structure of the IL-6R was determined in 1988 and found to consist of a 60-kDa binding protein (IL-6Rα) and a 130-kDa signal-transducing subunit (gp130) (164). The IL-6Rα contains both an Ig domain and a WSXWS motif characteristic of receptors that interact with cytokines sharing a four α-helical folding pattern (165). The signal-transducing subunit, gp130, also contains the WSXWS motif, but is not specific for IL-6 and can interact with other cytokines and polypeptides as well (166). Clustering of the gp130-chain by interactions with cytokine and cytokine specific binding protein (α-chain) is thought to trigger signaling (167, 168). The gp130 protein is ubiquitously expressed on various target cells (169). The IL-6R was shown to transmit the signal even if the intracellular portion was removed (167), showing that this internal part is not essential. An extreme example of this is provided by the brain-specific ciliary neurotropic factor (CNTF), which is essential for the survival of motor-neurons (170, 171). Its receptor closely resembles the IL-6R on the outside of the cell but has nothing at all inside. It connects directly with the cell membrane by means of a phosphatidyl inositol linkage.

Concluding Remarks

B cells are generated from pluripotent hematopoietic stem cells in bone marrow that also generate myeloid and erythroid elements. Decisive commitment to the B-cell lineage is championed by V-D-J recombination that brings V,

D, and J segments of IgH genes together to create a complete immunoglobulin gene. Pro-B cells, the earliest stage of B-cell development, contain the germ-line pattern of IgH genes and express characteristic B-cell lineage surface phenotypes. Pro-B cells can differentiate into pre-B cells accompanied by the rearrangement of IgH genes and expression of cytoplasmic μ-chains. Pre-B cells proliferate, rearrange L-chain genes, and express surface IgM giving rise to mature B cells. Further proliferation and differentiation of mature B cells into Ig-producing cells require T_H cells and cytokines. Various cytokines are involved in B-cell development and triggering.

Many cytokines involved in the regulation of B-cell development, activation, proliferation, and differentiation have been molecularly cloned. The studies with recombinant cytokines clearly demonstrate that the functions of the cytokines are not specific to B lineage cells as originally expected but mediate a wide variety of biological activities on various target cells and tissues. Examples of B-tropic multifunctional cytokines—IL-7, IL-4, IL-5, and IL-6—have been discussed in this review. Sites of action of each cytokine are not easy to delineate, and it is often not clear which cytokines induce B-cell proliferation and which ones induce differentiation of activated B cells for Ig secretion.

IL-7 plays an important role in the early development of B cells, particularly progenitors for B-2 cells as well as T cells. IL-7 may be involved in the rearrangement of Ig light chain genes. IL-7 has been also shown to be involved in the thymocyte differentiation. IL-4 plays a critical role in the development of both B and T cells, and in isotype-switching, particularly for IgE production by activated B cells. However, it is not clear whether IL-4 by itself is sufficient for isotype-switching. IL-4 is a key cytokine with respect to distinguishing T_H1 and T_H2 cells.

IL-5 is an important cytokine for the development and growth of B-1 cell progenitors and differentiation of mature B-1 and B-2 cells into Ig-secreting cells. I would particularly like to emphasize the importance of IL-5 in IgM and IgA secretion. IL-5 is a potent cytokine for inducing the production of natural antibody and antigen-induced IgA production. IL-5 is also a priming factor of B cells for other cytokines. In other words, IL-5 can synergize with many cytokines to enhance antibody production. If we compare T-cell activities of IL-5 with those of IL-7, IL-4, and IL-6, IL-5 is very weak and not attractive. At least in the murine system, IL-5 may be a B-tropic cytokine for lymphoid progenitor cells. IL-6 is a cytokine to induce terminal differentiation of B cells and enhance Ig production by activated B cells. Since IL-6 induces the proliferation of myeloma cells, it might enhance survival of plasma cells or induce one-round proliferation of plasma cells, resulting in enhanced Ig production.

There is no doubt about the involvement of these cytokines in B-cell proliferation and differentiation, alone or in concert with other B-tropic cytokines. Precise mechanisms and sites of action of each cytokine will be more precisely elucidated in the near future. Aberrant expression of receptors and signal transduction for IL-4, IL-7, IL-5, and IL-6 results in impaired B-cell responses. Overexpression of the cytokine genes may enhance the production of autoantibody.

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