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MINIREVIEW

B-Cell Growth and Differentiation Factors (42732A)

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I. Introduction. The system of the immune response to antigen is regulated by a series of interaction among B cells, T cells, and probably macrophages. Each B cell is genetically committed to become an antibody (immunoglobulin, Ig)-secreting cell against a distinct antigen epitope (1), and the antibody produced plays a key role in neutralizing relevant pathogenic microorganisms or in eliminating unnecessary molecules in the body.

It was shown that a T cell responding to, and specific for, the same antigen molecule was necessary for the B-cell response to that antigen (2–5). Two models were proposed to account for T-cell requirement for B-cell triggering: (a) that the T-cell help was delivered by a direct cell contact with B cells, and (b) that the T cell could produce a factor which acted, only at short range, on a receptor on the B-cell surface (6). There is now evidence that helper T cells recognize antigen in the context of class II major histocompatibility complex (MHC) molecules on accessory cells and/or B cells (7). It seems clear that the above two models represent two different mechanisms, and there is a consensus among many investigators that the direct T-cell–B-cell interaction is essential for the initial B-cell activation in the physiological environment of the whole animal. In the factor model, T cells can be activated by cell contact with B cells to produce an antigen nonspecific soluble factor in the vicinity of the B cell, and the B cell could respond in the absence of the T cell if a sufficient amount of the factor is provided. Actually, there is abundant evidence for a variety of T-cell- and macrophage-derived factors which act on B cells. These factors are non-antigen-specific, MHC unrestricted and are able to exert a variety of different functions.

In the early studies of Dutton and associates it was assumed that there was a single T-cell-derived factor (6), and the activity was designated as T-cell-replacing factor (TRF) (8). They measured the number of primary anti-sheep red blood cell (SRBC) plaque-forming cells (PFC) in a T-cell-depleted B-cell population after 4 days of culture with SRBC and helper factor. Kishimoto and Ishizaka showed a similar requirement for antigen or anti-Ig and T-cell factors in the secondary anti-hapten response in the rabbit (9, 10). In these studies, T-cell-depleted populations retained a considerable percentage of non-B cells including residual T cells and macrophages which may produce B-cell stimulatory factors (BSFs). Furthermore, investigators realized that the source of TRF, T-cell-derived supernatants (Sup), contains various factors including T-cell growth factor (interleukin 2) and lymphocyte-activating factor (interleukin 1), both of which can induce B-cell growth and differentiation.

In further studies, investigators employed many strategies to minimize the above problems. The first of these was the use of B-cell tumor populations which provide a much more homogeneous population of B cells (11–13). Second, attempts were made to use

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antibody to the immunoglobulin receptor rather than antigen (10, 14). A third approach was to use culture supernatants of certain cloned T-cell lines and hybridomas selected for their ability to produce higher titers of activity and to subject further these Sup to physicochemical procedures to separate one activity from another.

In later studies, it was demonstrated that T-cell-dependent B-cell activation involves a series of discrete stages including both a proliferative phase and a separate state of differentiation (15–18). Series of experiments have been explored to characterize the nature of T-cell-derived BSFs that can induce B-cell growth and differentiation, and to clarify the precise roles of BSFs in the B-cell development. However, the precise number of BSFs is currently unclear, and elucidation of each BSF is hampered by the pluripotency of some BSFs, by the synergistic and inhibitory interactions between BSFs, and by the complex of various BSF bioassays. The increasing availability of cytokines prepared by recombinant DNA technology, and the more extensive use of defined assay systems, now allow a more precise delineation of the bioactivities of BSFs on B cells.

BSF-1, IL-1, IL-2, and BCGF II have been claimed to act as B-cell growth factor, BCGF. A number of factors have been also found to act in B-cell differentiation. These include TRF, BCGFII, BSF-2/IL-6, IL-1, and IL-2. Some lymphokines were shown to act on activated B cells as a B-cell growth and differentiation factor. There is no doubt that advanced research on IL-1 and IL-2 strongly influenced the developments that led to the elucidation of B-cell growth and differentiation factors. However, it is still controversial whether IL-1 or IL-2 plays an essential role for B-cell growth and/or differentiation.

The cDNA for three distinct BSFs has recently been cloned: (i) BSF-1/IL-4, which causes B-cell activation, growth, and differentiation; (ii) TRF/BCGF II/IL-5, which induces B-cell growth and differentiation with diverse activities; and (iii) B-cell differentiation factor, BSF-2/IL-6, which induces differentiation of B cells without inducing cell proliferation. The present article deals with the molecular properties and biological functions of these factors. Since reviews on BSF-1/IL-4 (19) and on BSF-2/IL-6 (20) have been published, I mainly focus on the molecular and functional properties of TRF/BCGF II/IL-5 and on the prominent features of BSF-1/IL-4 and BSF-2/IL-6.

II. BSF-1/IL-4 as a B-Cell Activation, Growth, and Differentiation Factors. Murine BSF-1/IL-4 is a T-cell-derived glycoprotein first described by Howard *et al.* (21) as a B-cell growth factor for highly purified small B cells stimulated with suboptimal doses of anti-IgM antibody ($\sim$5 μg/ml). An essential feature of these experiments was the use of highly purified B-cell populations and low numbers of cells per culture (as few as 5 $\times$ 10^4) in order to minimize the contribution from contaminating non-B cells. This activity was identified in the Sup from phorbol myristate acetate (PMA)-stimulated EL4 thymoma cells, which has an apparent mol wt of 18 kDa. The factor was initially designated BCGF, and was subsequently renamed BCGF I (22), since the presence of two distinct factors with BCGF activity has been demonstrated, namely BCGF I and BCGF II. BCGF I was then designated BSF-1 on the basis of a discussion at the nomenclature meeting (23), and most recently has received the designation interleukin 4 (IL-4) (24, 25). BSF-1 is found in numerous T-cell sources, including Sup from alloreactive T-cell clones, induced or nonstimulated Sup from T-cell hybridomas, and Sup from some, but not all, antigen-induced long-term normal T-cell lines (21, 26–30). Extensive studies on the molecular and immunological characterization of BSF-1 have been carried out (31, 32). BSF-1 has been purified to homogeneity and a monoclonal antibody was prepared against BSF-1 (33). N-terminal amino acid sequences of 20 residues have been reported (34). These sequences are in agreement with that inferred from the nucleotide sequence of the BSF-1 cDNA (24, 25).

Initially BSF-1 was thought to be a "growth factor" that acts early in G_1 phase to synthesize DNA (21). However, experimental results indicated that BSF-1 acts on resting (G_0 phase) B cells in the absence of any costimulant: (i) BSF-1 induces an 8- to 10-fold increase in class II (Ia) MHC antigen expression on resting B cells (35, 36), (ii) BSF-1 causes a small but significant increase

in cell volume of resting B cells (37), and (iii) pretreatment of resting B cells with BSF-1 alone for a period of 24 hr promoted subsequent entry of cells into S phase and their subsequent response to anti-IgM and BSF-1 by about 12 hr (38, 39). All of these results indicate that BSF-1 is multifunctional and exerts its action on resting B cells. It is not a simple growth factor but, rather, an "activation factor."

BSF-1 also functions as a differentiation factor of B cells in certain situations. In general, murine B cells treated with LPS will secrete IgM and IgG_3, but little or no IgG_1. It was shown, however, that the addition of Sup containing BSF-1 results in the secretion of IgG_1 and the partial suppression of IgG_3, IgG_{2b} production. It has little to no effect on IgM production (26–28). The factor mediating this activity was originally designated B-cell differentiation factor for IgG_1 (BCDFγ) (26), or IgG_1 induction factor (27, 28). Furthermore, it was demonstrated using Northern blot analysis that surface IgG_1-negative B cells cultured with Sup containing BCDFγ showed an increase in the levels of steady-state γ1-mRNA expression with a concomitant decrease in γ2b- and γ3-mRNA levels (40, 41), suggesting that BCDFγ is an isotype "switch" factor. The biochemical characterization of IgG_1-induction factor indicated that it was similar to BSF-1 in both its molecular mass and its isoelectric point (31). Subsequently, a monoclonal anti-BSF-1 antibody was shown to inhibit the activity of purified BCDFγ, confirming that BSF-1 and BCDFγ are the same molecule (42). Purified BSF-1 from D9.1 cells could induce enhanced IgE production of LPS-stimulated B cells (43).

Noma *et al.* (24) cloned the cDNA for BSF-1 from a cDNA library derived from a IgG_1-induction factor-secreting murine T-cell line 2.19 using the SP6 vector system by monitoring the ability of the expressed product to stimulate LPS blasts to secrete IgG_1. Translation products of the cDNA insert induces (i) an increase in IgG_1 secretion and a concomitant decrease in the levels of IgG_{2b} and IgG_3 isotypes, (ii) DNA synthesis in resting B cells in the presence of anti-Ig antibodies, and (iii) increased expression of Ia antigen on small B cells. Sideras *et al.* (44, 45) studied the expression of BSF-1 mRNA in different cellular populations. BSF-1-specific mRNA was expressed in one out of 300 spleen cells upon the mitogenic stimulation.

Lee *et al.* (25) independently cloned cDNA for BSF-1. They originally selected the cDNA encoding for activity of mast cell growth factor-2 (MCGF-2) and T-cell growth factor-2 (TCGF-2), which is distinct from IL-2. They found that the Sup of COS-7 cells transfected with the cDNA not only had MCGF-2 and TCGF-2 activities, but also exerted the capacity to induce the proliferation of anti-IgM-stimulated B cells, to induce Ia antigen on resting B cells, and to induce IgG_1 and IgE secretions by LPS-stimulated B cells (25). The latter activity is really BSF-1 activity. It was also demonstrated that recombinant BSF-1 induces growth and differentiation of PMA-stimulated intrathymic T-cell precursors from fetal mice (46), and induces growth of mast cells from normal connective tissues (47). cDNA cloning studies demonstrated that BSF-1 has a wide variety of functions as shown in Table I. Since the function of BSF-1 is not restricted to B cells, the proposed name is IL-4.

The BSF-1/IL-4 cDNA codes for 140 amino acids with the signal sequence of 20 residues. The secreted core polypeptide contains 120 residues with a molecular mass of 14, 137 (24, 25). The BSF-1 sequence shows some homology in limited regions with granulocytes–macrophage–colony–stimulating factor (GM–CSF) and interferon-γ (IFNγ), suggesting a distant molecule from these lymphokines. Yokota *et al.* (50) have obtained a clone from a human cDNA library, prepared from Con A-stimulated cells of the human T-cell line 2F1. It showed 50% DNA sequence homology with murine BSF-1/IL-4 at the level of amino acid sequence and little to no significant homology with other human B-cell growth factor. The recombinant human BSF-1/IL-4 could induce proliferation of anti-IgM-stimulated B cells and of human T-cell clones (50, 51). Intriguingly, it was also demonstrated that BSF-1/IL-4 induced the expression of $Fc_\epsilon RII$ on B cells (52, 53). Since $Fc_\epsilon RII$ is believed to be involved in the regulation of IgE synthesis, BSF-1/IL-4 may be involved in the regulation of immediate-type hypersensitivity in

TABLE I. BIOLOGICAL PROPERTIES OF RECOMBINANT IL-4

	Target cells	References
1. Induction of DNA synthesis and growth of purified B cells stimulated with anti-Ig antibodies (BCGF I)	B	24, 25, 48
2. Induction of increase in the levels of class II MHC antigen expression on the surface of resting B cells	B	24, 25
3. Induction of increased expression of $Fc_\epsilon RII$ on the surface of resting B cells	B	52, 53
4. Preparations of resting B cells for entry into S phase	B	38, 39
5. Induction of IgG_1 and IgE secretion ($BCDF_\gamma$ and $BCDF_\epsilon$) and suppression of the IgG_3 and IgG_{2b} in LPS-stimulated B cells	B	24, 25
6. Induction of DNA synthesis by certain IL-2-dependent T-cell lines (TCGF-II)	T	25, 48, 50
7. Induction of growth and differentiation of intrathymic T-cell precursors from fetal mice	T	46
8. Induction of growth of IL-3-dependent mast cell lines (MCGF-II) as well as normal connective tissue-type mast cells	M	24, 47
9. Induction of macrophage enhancement of antigen presenting ability	M	49

two different ways: one is as BCDF for IgE secretion and the other is as inducers of $Fc_\epsilon RII$ receptor expression.

Since the recombinant BSF-1/IL-4 is available with homogeneity, it is now possible to define the cellular receptors for BSF-1/IL-4. It was reported that a single class of high-affinity of receptors for BSF-1/IL-4 has been detected on several types of cells of hematopoietic derivation including B cells. The number of receptor molecules on resting B and T cells was estimated to be 500 per cell (54, 55).

III. TRF/BCGF II/IL-5 as a B-Cell Growth and Differentiation Factor. *(a) TRF as a B-cell growth and differentiation factor.* The studies on IL-5 began in the early 1970s when we described a T-cell-derived, antigen non-specific B-cell differentiation factor which we called TRF. TRF activity was found in the Sup of $L3T4^+$ T cells from *Mycobacterium tuberculosis* (Tbc)-primed mice that had been stimulated with purified protein derivative (PPD)-presenting cells (56, 57). The establishment of a TRF-producing T-cell hybrid B151K12 (B151) by means of fusion between Tbc-primed T cells and murine thymoma BW5147, which does not secrete any detectable levels of other BSFs, enabled us to demonstrate that TRF is a lymphokine distinct from IL-1, IL-2, IL-3, BSF-1/IL-4, and IFNγ (58, 59). TRF activity was initially assessed by using extensively T-cell-depleted splenic DNP-KLH-primed B cells as differentiation-inducing activity to anti-DNP IgG antibody-secreting cells. *In vivo* growing murine chronic B-cell leukemia, BCL_1, cells were then shown to differentiate into IgM-secreting cells by stimulation with TRF-containing B151 Sup (12). Therefore, we started to purify TRF-active molecules employing two assay systems for TRF activity: (a) induction of anti-DNP IgG PFC responses in cultures of splenic B cells from DNP-KLH-primed BALB/c mice, and (b) induction of IgM PFC from BCL_1 cells. The TRF purified from B151-Sup to homogeneity was shown to be an acidic glycoprotein with a molecular mass of 50 to 60 kDa on gel permeation chromatography, and it migrated into 18 kDa on SDS–PAGE under reducing conditions (60). The purified TRF showed undetectable IL-1, IL-2, IL-3, BSF-1/IL-4, and IFNγ activities. It showed, however, growth-promoting activity of BCL_1 cells as well as dextran sulfate (DXS)-stimulated normal B cells (BCGFII) (22). BCGF II activity always resided in the same fraction in which TRF activity was detected (61), suggesting that a single molecule (HPLC-purified B151-TRF) is able to induce B-cell growth and differentiation. This was further substantiated by the fact that monoclonal antibody against B151-TRF can inhibit both activities of HPLC-purified B151-TRF as follows (62).

The spleen cells from Wistar rats which had been immunized with HPLC-purified B151-TRF were fused with the P3-X63-Ag 8.653 mouse myeloma using polyethylene

glycol. Cells from one fusion well which had reproducible inhibitory and absorptive activities of HPLC-purified B151-TRF were subjected to a series of subclonings and produced two lines, TB13 and NC17, both secreting rat IgG_1 antibodies, which possessed identical properties and were used interchangeably (62). Both TB13 and NC17 block TRF-mediated anti-DNP IgG response of DNP-primed B cells as well as the IgM secretion of BCL_1 cells. Moreover, they also inhibited proliferation of BCL_1 cells induced by B151-TRF. However, the activities of IL-1, IL-2, IL-3, and BSF-1/IL-4 were not inhibited by TB13 even at 10 μg/ml.

Monoclonal anti-TRF antibody TB13 could also be used for purification of TRF from B151-Sup. When we applied 3 liters of B151-Sup containing 30,000 units of TRF to the TB13-coupled affinity column, less than 100 units of TRF appeared in the effluent, and elution with acetic acid yielded 28×10^3 units. Eluted materials have a molecular mass of 46 kDa by SDS–PAGE analysis (62) under a nonreducing condition and migrated to 23 to 26 kDa under a reducing condition, suggesting that B151-TRF comprises a dimer form. Recently, affinity-purified B151-TRF was further purified by reversed-phase HPLC column, and its partial N-terminal acid sequence was determined (unpublished). The results revealed that a single amino acid sequence of 27 residues is in agreement with the amino acid sequence inferred from the nucleotide sequence of TRF cDNA clone derived from Kinashi *et al.* (63).

(b) BCGF II as a B-cell growth and differentiation factor. The existence of a second T-cell-derived factor active on murine B cells and distinct from BSF-1/IL-4 was initially suggested by Swain and Dutton (13). They identified a growth-promoting activity in the Sup derived from the alloreactive T-cell line C.C3.11.75, commonly designated DL, which stimulated *in vitro* proliferation by *in vivo* passaged BCL_1 lymphoma cells, and by DXS-stimulated B cells (22), and they called the activity (DL)BCGF. BCGF I (BSF-1) showed no activity in these assays, and DL-Sup had no activity in the anti-IgM costimulator assay described by Howard *et al.* (21). Such studies clearly demonstrated the existence of two distinct factors, called BCGF I and BCGF II (22). Soon after that, Swain *et al.* (64, 65) found that their BCGF II could induce Ig-secretion of activated B cells and the differentiation activity copurified with the proliferative activity in a variety of chromatographic separations. Similarly, Pike *et al.* (66) in a antigen-specific single-cell assay showed that Con A-stimulated EL4-Sup contained a proliferation and differentiation activity .

Recently, a novel activity of BCGF II has also been reported by Sanderson *et al.* (67). Using Sup derived from alloreactive T-cell clones and hybrids, they have identified an eosinophil differentiation factor (EDF) which appeared to additionally display BCGF II activity. EDF was titrated by incubating bone marrow cells derived from *Mesocestoids corti*-infected mice with test samples, and by measuring eosinophil development at 5 days by a colorimetric assay for eosinophil peroxidase. Gel filtration chromatography revealed an apparent molecular mass of 45 kDa for both activities. A T-cell hybrid, NIMP-TH1, has recently been described which secretes both BCGF II and EDF active molecules. The BCGF II and EDF produced by these cells were copurified in every fractionation procedure employed: both activities are associated with a protein with an approximate molecular mass of 44 kDa and a p*I* of 5.0 (68). NIMP-TH1 T cells did not produce IL-1, IL-2, IL-3, IFNγ, or IL-4, immediately distinguishing EDF from these lymphokines. EDF had no effect on purified resting B cells as measured by thymidine uptake, whereas it could induce DNA synthesis as well as Ig secretion by naturally occurring large B cells (68).

The discovery that BCGF II induced both growth and differentiation of activated B cells prompted speculation that this factor might be identical to a number of B-cell stimulatory activities identified using other B-cell assays. In particular, TRF-active molecules produced by B151K12 could be identical to BCGF II on the basis of evidence described in the previous section. As described below, TRF, BCGFII, and EDF activities were found to be mediated by a single molecule.

(c) Cloning of complementary DNA for TRF/BCGF II. The complementary DNA

(cDNA) encoding for murine TRF/BCGF II active molecules was isolated from poly(A)$^+$ RNA of 2.19 T cells (63). The RNA was size-fractionated on a sucrose density gradient. Aliquots of fractionated mRNA were microinjected into *Xenopus* oocytes, and secreted products were bioassayed for TRF and BCGF II activities with a BCL_1 cell line. cDNA libraries from poly(A)$^+$ RNA and from the enriched mRNA by the sucrose density fractionation were constructed using SP6-K vector system (24). A DNA mixture of 5×10^4 independent cDNA clones was cleaved with *Sal*I to linearize plasmid DNA, and mRNAs were synthesized using SP6 RNA polymerase. The synthesized RNAs were microinjected into *Xenopus* oocytes, and the translation products secreted into the incubation medium were assayed for TRF and BCGF II activities. Pools that tested positive in the assay were further divided into smaller pools, that were tested under the same procedures until a single cDNA clone capable of encoding for TRF and BCGF II was obtained. One clone, clone 23, was selected positive for BCL_1 proliferation and IgM PFC response and was termed pSP6K-mTRF23 (63).

The nucleotide sequence of the entire TRF cDNA was determined as described (63). The TRF cDNA codes for a polypeptide of 133 amino acids which contains the N-terminal signal sequence of 20 residues. The secreted core polypeptide has a relative molecular mass of 12.4 kDa. Three possible N-glycosylation sites as well as three cysteine residues are present in the polypeptide sequence. The deduced amino acid sequence of TRF does not show extensive homology with known proteins including lymphokines except for short segments of murine IL-3, murine GM-CSF, and murine IFNγ.

Azuma *et al.* (69) cloned the cDNA for human TRF from poly(A)$^+$ mRNA extracted from ATL-2 cells using the murine TRF cDNA clone as a probe. The isolated cDNA clone encodes 134 residues including a signal sequence of the N-terminal 19 hydrophobic residues (69). The nucleotide and amino acid sequence of the coding regions of the human and murine TRF cDNA are 77 and 70% homologous, respectively. Human TRF induces Ig secretion of SAC-stimulated peripheral B cells.

Recently, Yokota *et al.* (70) isolated a mouse cDNA clone that expresses IgA-enhancing factor (IgA-EF) and eosinophil colony-stimulating factor (Eo-CSF) activities from Con A-activated cloned T cells ($Ly1^+2^-$/9). They also isolated a human cDNA clone encoding for an interleukin with IgA-EF and Eo-CSF activities from a human cloned T cells. Their DNA sequence analysis revealed that mouse and human cDNA clones encode for 133 and 134 amino acids, respectively (70), and are identical to the cDNA clone encoding for TRF and BCGF II active molecules.

The products of the TRF mRNAs transiently translated in the rabbit reticulocyte lysates have a molecular mass of apparently 14 kDa (71). By contrast, the rTRF/IL-5 translated in *Xenopus* oocytes has apparent molecular mass of 45 to 50 kDa, and migrates to a molecular mass of 25 to 30 kDa under the reducing condition, indicating that mature rTRF also consists of dimer forms (71). Coinjection of tunicamycin and TRF mRNA into *Xenopus* oocytes induced the production of 27- to 28-kDa dimer molecules which exert TRF and BCGF II activities, suggesting that the N-linked carbohydrate moiety derived by N-glycosylation may not play an essential role in the biological activity of TRF (unpublished). The rTRF was purified using immunoaffinity gel coupled with anti-TRF antibody, and its N-terminal amino acid sequence was determined. N-terminal methionine in secreted TRF was found at position 21 of the amino acid sequence predicted from the cDNA pSP6K-mTRF23.

Various biological activities of rTRF were then tested. The rTRF stimulates DNP-KLH-primed B cells in the presence of DNP-ovalbumin, and augments anti-DNP IgG response (63, 71). These properties of rTRF agree with the criteria of TRF derived from B151. The rTRF also augments [^{3}H]thymidine incorporation into B cells stimulated with DXS, strongly supporting our original observation that TRF and BCGF II activities belong to a single molecule. The rTRF has no IL-1, IL-2, IL-3, or BSF-1/IL-4-like activity (71) but acts on a number of target cells to induce growth and/or differentiation (Table II). Because of diverse activities and targets, we proposed that

TABLE II. BIOLOGICAL FUNCTIONS OF RECOMBINANT IL-5

	Target cells	References
1. Inductions of differentiation of activated normal B cells and murine chronic B-cell leukemia (BCL_1) cells into Ig (IgM, IgG, or IgA)-secreting cells (TRF, IgA-EF)	B	63, 70, 71
2. Induction of increased expression of secretory forms of μ- and α-mRNA ($BCDF\mu$, $BCDF\alpha$)	B	72, 80
3. Induction of DNA synthesis of DXS-stimulated normal B cells and BCL_1 cells (BCGF II)	B	63, 72
4. Induction of up-regulation of the functional IL-2 receptor expression on B cells	B	73, 86, 87
5. Augmentation of cytotoxic T-cell generation in antigen-stimulated thymocytes conjunction with IL-2 (KHF)	T	92
6. Enhancement of the IL-2 receptor expression of T cells	T	92, 93
7. Induction of growth and differentiation of eosinophil (EDF, Eo-CSF)	Eo	70, 94, 95

TRF or BCGF II be called interleukin 5 (IL-5). Hereafter, I will refer to TRF or BCGF II as IL-5.

(d) Functional aspects of recombinant IL-5 in the B-cell response. As mentioned in the previous section, the IL-5 induces IgM synthesis in BCL_1 cells, in *in vivo* activated B cell-blasts, and in anti-IgM and BSF-1/IL-4-stimulated B cells. The IL-5 can induce terminal differentiation of DNP-primed B cells into anti-DNP responses in IgM, IgG, and IgA classes (63, 71, 72). The activity of IL-5 for IgA induction was also inhibited by anti-TRF monoclonal antibody. A selective enhancing effect of IL-5 on IgA secretion was also reported by Yokota *et al.* (70), as described.

The IL-5 can also augment primary B-cell response to antigen in the absence of T cells. For instances, IL-5 stimulates nonprimed B cells and augments anti-SRBC IgM response in the presence of SRBC (73, 74). Alderson *et al.* (75) have provided convincing evidence that the effects of IL-5 are mediated by direct action of the lymphokine on B cells. They cultured individual B cells in Terasaki plate culture dishes. They demonstrated that the addition of IL-5 in the culture of single fluorescein (FLU)-specific B cells and FLU-polymerized flagellin markedly increased the frequencies of both proliferating clones and antibody-secreting clones to FLU. However, IL-5 exerted little activity when acting alone.

It has long been debated and is still controversial whether lymphokine can induce maturation of resting B cells to Ig-secreting cells without proliferation, i.e., for B-cell maturation factor (BMF) activity. Karasuyama and his associates demonstrated that IL-5 has BMF activity on purified resting B cells from BALB/c *nu/nu* mice (76).

Proliferation-inducing activity of IL-5 could induce growth of B cells represented by BCL_1. The experiments described by Melchers and his associates may give us more information on this issue. They reported that T-cell-derived B-cell replication factor which they called β-factor acted late in the cell cycle in the G_2 phase, causing proliferation of B cells which had been stimulated with anti-IgM antibody and macrophage-derived α-factor (77, 78). Their recent analysis using recombinant interleukins revealed that IL-5 exerts β-factor activity and promotes the progression of activated B cells through the cell cycle (76, 79), indicating that IL-5 plays an essential role in the B-cell growth at a certain stage of every cell cycle.

It is still not fully understood whether IL-5 influences the expression of specific Ig isotypes in B cells. Matsumoto *et al.* (80) demonstrated that BCL_1 or resting B cells cocultured with IL-5 expressed increased levels of secreted forms of μ-mRNA. The B cells cultured with LPS plus IL-5 expressed increased levels (4-fold) of the secreted as well as membrane forms of IgM and 2.5-fold increase of secreted forms of γ1-mRNA. With regard to augmentation of γ1-mRNA expression, BSF-1/IL-4 was approximately 4-fold higher than that of IL-5. Purified DNP-primed B cells cultured with antigen in the presence of IL-5 also expressed a 4-fold increased level of μ-mRNA and at least a 2-fold increased level of γ1- as well as α-mRNA (72, 80), suggesting that IL-5 preferentially induces an increase in the levels of μ-mRNA secreted and substantially increases the level of α-mRNA.

IL-5 may be a "maturation" factor for B cells rather than a "class-switch" factor.

The expression of cell-surface receptors for IL-2 by activated T cells has long been established (81), and under appropriate conditions B cells can also express receptors for and respond to IL-2 (82). To achieve high numbers of receptors on normal B cells some form of activation of the cell is required (83–85). We found that suboptimal doses of IL-5 could synergistically stimulate BCL_1 cells or DNP-primed B cells with IL-2 for their maximum differentiation into Ig-secreting cells (59), and its effect was completely inhibited by anti-IL-2 receptor antibody. Direct binding assay of ^{125}I-labeled IL-2 to DNP-primed B cells which had been cultured with IL-5 revealed that IL-5 induced the expression of two classes, high- and low-affinity, of receptors for IL-2, and the average numbers of high- and low-affinity IL-2 binding sites per IL-5-activated DNP-primed B cell were 400 and 6500, respectively (73). Dissociation constants obtained were 57.1 p*M* for high-affinity and 8.13 n*M* for low-affinity receptors. Furthermore, IL-2 receptors induced were functional because IL-2 can induce differentiation of those B cells into Ig-secreting cells. Intriguingly, IL-5 could induce increases in the levels of steady-state mRNA expression for IL-2 receptor by approximately eight-fold (73). Nakanishi *et al.* (86) found up-regulation of IL-2 receptor on a monoclonal B cells (BCL_1-CL-3) by IL-5. Loughnan *et al.* (87) recently reported that B cells cultured with Sup from Con A-stimulated EL4 cells with or without LPS displayed IL-2 receptors. This bioactivity of EL4-Sup could not be replaced by any concentration of BSF-1/IL-4, IL-1, IL-2, or IL-3 tested. However, IL-5 was a potent inducer of IL-2 receptor expression. The receptors so induced appeared to be functional because receptor-expressing blasts responded to IL-2 by proliferation.

IL-5 is the first lymphokine molecularly identified as IL-2 receptor-inducing factor. IL-2 receptors induced are functional and probably of physiological importance in an on-going immune response. It is not clear, however, whether IL-5 can induce preferential expression of p75 or p55 of IL-2 receptor, and whether every B cell responsive to IL-5 can express IL-2 receptors.

(e) IL-5 mRNA expression. Since the original description of TRF, TRF-active substances have been reported by many investigators to be in Sup of cloned T cells or T-cell hybridomas. However, until now there was no concrete evidence that each investigator was describing the same active molecule. Availability of monoclonal anti-IL-5 antibody and IL-5 cDNA enabled us to reexamine IL-5-producing cells and mechanisms of IL-5 gene expression.

Expression of IL-5 mRNA in T-cell lines, macrophage–monocyte cell line, myelomonocytic cell line, BCL_1, or myeloma cells was examined by Northern blot analysis of poly(A)$^+$ RNA using IL-5 cDNA as a probe, and the results revealed that RNA from constitutively TRF-producing B151, but not from other cell lines, hybridized with a single 1.7-kb band (71). RNAs from neither mastocytoma nor fibroblastic cell lines showed hybridization with IL-5 cDNA. The expression of IL-5 mRNA in B151 was augmented by stimulation with PMA plus calcium ionophore, by which TRF production was augmented (88). Stimulation of EL4 and D9 cells with PMA and Con A, respectively, induced an increase in the levels of IL-5 mRNA expression accompanying IL-5 production (71). These results suggest that IL-5 may be produced predominantly by T cells and its gene expression is inducible, although T-cell subsets producing IL-5 are still not clear.

The Northern blot analysis is also revealed that 1.7-kb RNA from PPD-stimulated, Tbc-primed cells hybridized with IL-5 cDNA. Normal spleen cells stimulated with Con A showed a significant, but approximately three-fold less, expression of IL-5 mRNA. An undetectable level of IL-5 mRNA expression was observed in PMA plus calcium ionophore-stimulated spleen cells. Interestingly, hybridization of RNA from Con A-stimulated spleen cells with IL-2 cDNA was much stronger than that from PPD-stimulated, Tbc-primed cells (71). This may imply that IL-5-producing T cells comprise T-cell subpopulation distinct from those for IL-2-producing T cells, although other possibilities are not excluded.

(f) Actions of IL-5 on cells of non-B-cell lineage. Induction of CTL from thymocytes requires T-cell-derived soluble mediators

that have been operationally referred to as a killer-helper factor (KHF) (89–91). The KHF induces generation of CTL from CTL precursors in peanut agglutinin-binding (PNA^+) thymocytes in the presence of stimulator cells in conjunction with IL-2. KHF is shown to be distinct from IL-1, IL-2, IL-3, BSF-1/IL-4, and IFNγ (91).

Since IL-5 can induce functional IL-2 receptors on resting as well as activated B cells, KHF activity in Sup from six IL-5-producing T-cell hybrid clones was tested (92). Significant KHF activities were detected in Sup from various IL-5-producing T-cell hybrids including B151. The only exception was the 2Y4 Sup, which exerted KHF activity without showing detectable IL-5 activity. IL-5 and KHF active molecules had identical elution profiles in every purification step. KHF activities of HPLC-purified B151-TRF were completely inhibited by anti-IL-5 antibody. The IL-5 also exerted KHF activity, and its activity was completely suppressed by rat monoclonal anti-IL-5 antibody (92), indicating that IL-5 acts on premature T cells concomitant with the presence of antigen and IL-2. Even though the IL-2 receptor-inducing activity of IL-5 may account in part for the CTL-inducing activity of IL-5 (92, 93), the exact mechanism by which IL-5 mediates CTL-inducing activity remains unclear.

EDF has originally been defined as an activity that stimulated the production of functional eosinophils from bone marrow in the liquid culture system (67). However, it is not clear whether this factor is specific for the eosinophil differentiation, or whether it is analogous to CSFs described for other hematopoietic lineages. Using the clonal cell culture and liquid culture system, we studied the *in vitro* effect of IL-5 on murine hematopoietic cells at various stages of differentiation (94). IL-5 alone acted on untreated bone marrow cells and supported the formation of a small number of colonies, all of which were predominantly eosinophilic. However, it did not support colony formation by spleen cells from 5-fluorouracil (FU)-treated mice, in which only primitive stem cells had survived. Eosinophil-containing colonies were formed from these cells in the presence of IL-5 and G-CSF together. In contrast, G-CSF alone did not support any eosinophil colonies. From a small number of replated blast cells (enriched hemopoietic progenitors), eosinophil colonies were induced in dishes containing IL-5 but not in those containing G-CSF alone. From these findings, it was concluded that IL-5 did not act on primitive hematopoietic cells, but on blast cells induced by IL-3 or G-CSF. IL-5 specifically facilitated the terminal differentiation and proliferation of eosinophils. Moreover, IL-5 could be effective in maintaining the viability of mature eosinophils obtained from peritoneal exudate cells of mice infected with parasites (94). The synergistic effect of IL-5 and colony-stimulating factors on the expansion of eosinophiles is supposed to contribute to the urgent mobilization of eosinophils at the time of helminthic infections and allergic responses.

The property of T-cell-produced IL-5 for differentiation of eosinophils was suggested by Sanderson *et al.* (67), as described. Eosinophil colony-stimulating activity of human as well as murine IL-5 on human cord blood was clearly shown by Campbell *et al.* (95) and Yokota *et al.* (70).

(g) Receptors for IL-5. The nature of the receptors for IL-5 on the cell surface has become a matter of interest. Direct measurement of IL-5 binding to receptors on cell surfaces has been made possible by the availability of large quantities of highly purified IL-5. The purified IL-5 was labeled with ^{125}I using Bolton-Hunter reagent. Cells for ^{125}I-IL-5 binding assay were washed and incubated in serum-free medium for at least 120 min at 37°C before assay. We used the IL-5-responding tumor line BCL_1-B20 as target cells for binding assay.

The binding of ^{125}I-IL-5 to BCL_1-B20 cells was in a saturable manner at 37°C and achieved a steady-state level within 10 min. Unlabeled TRF/IL-5 inhibited the binding of ^{125}I-IL-5 in a dose-dependent manner. As much as 90% of the total radioactivity of ^{125}I-IL-5 that bound to BCL_1 without competitor was inhibited by 50-fold excess unlabeled IL-5. No significant competition was observed with any other cytokines such as IL-1, IL-2, IL-3, GM-CSF, IFNγ, and BSF-1/IL-4 in a large excess. The binding of IL-5 to its receptor is also inhibited by the monoclonal anti-IL-5 antibody TB13 which is also a potent inhibitor of the biologic function of IL-5. The specific binding of ^{125}I-TRF/IL-5

to BCL_1-B20 was in a saturable manner. *Scatchard* plot analysis of the binding data revealed two linear regression lines, suggesting that there were two sets of binding sites on BCL_1-B20 (unpublished data).

IV. BSF-2/IL-6 as a B-Cell Differentiation Factor. The existence of B-cell differentiation factor (designated BCDF and subsequently BSF-2), which is involved in the final differentiation of B cells to Ig secretion without inducing cell growth, was originally demonstrated by Kishimoto *et al.* (16, 20, 96) and Kehrl *et al.* (97) who employed a human B-lymphoblastoid cell line that was responsive to BCDF. IgG secretion was induced in CESS cells within 48 hr after the addition of Sup from PHA-stimulated T cells. The presence of BCDF was confirmed by the establishment of BCDF-producing human T hybrid clones. Subsequently, an HTLV-I-transformed human T-cell line (TCL-Na1), which secreted relatively large amounts of BCDF, was established (98) and the Sup were employed for the isolation and purification of human BCDF. BCDF was then purified to homogeneity by employing sequential chromatographies, and its partial sequence of the N-terminal amino acids was determined (99). They prepared polyclonal antibody against synthetic peptide of N-terminal 13 amino acids of BCDF (100). The anti-peptide antibody-coupled affinity column could absorb BCDF activity of PHA-Sup. Therefore, they designated BCDF as BSF-2 (100).

The cDNA for BSF-2 has been cloned by probing cDNA library prepared from poly(A)$^+$ RNA of a BSF-2-producing TCL-Na1 cell line, with the use of the synthetic oligonucleotide mixtures, monitoring by induction of IgM secretion in SKW6-CL4 (101). The Sup of the BSF-2 cDNA transfected COS-7 cells exerted BCDF activity on pokeweed mitogen (PWM)-stimulated human peripheral B cells without showing growth-promoting activity (101). The cDNA encodes 212 amino acids including 28 strongly hydrophobic amino acid residues at N-terminus which is cleaved off during the processing. The sequence of BSF-2 was not very similar to human IL-1α, IL-1β, IFNα, IFNβ, IFNγ, GM-CSF, IL-3, BSF-1/IL-4, and TRF/BCGF II/IL-5, but it did have limited homology with human G-CSF.

Soon after publication of the paper by Hirano *et al.* (101), others recognized (102, 103) that the BSF-2 sequence is identical to that published for human 26-kDa protein (104) and IFN-β2 (105). Recombinant human 26-kDa protein exerted B-cell growth-modulating and differentiating activity (106). Almost simultaneously, Van Damme *et al.* (107, 108) showed that a potent B-cell hybridoma/plasmacytoma growth factor (HPGF) is produced by human fibroblast after treatment with IL-1, poly(I):poly(C), and/or cycloheximide. Furthermore, the N-terminal amino acid sequence of purified natural HPGF was identical to that of a stretch starting at amino acid 28 in the sequence of BSF-2, 26-kDa protein, and IFN-β2. These independent data support the notion that IFN-β2, HPGF, and human 26-kDa protein are identical to BSF-2.

Human recombinant BSF-2 was clearly demonstrated to exert growth-promoting activity on myeloma cells derived from patients (109). More interestingly, stimulation of fibroblasts with IL-1 induces an increase in the levels of steady-state mRNA expression for BSF-2 (106, 107). It was also reported that several tumor cells such as cardiac myxoma, cervical cancer cells, and bladder cell carcinomas also aberrantly produce large amounts of BSF-2 (101). On the basis of the diverse activities on different target cells, BSF-2 will be called interleukin 6 (IL-6) (20). Recombinant IL-6 also acts on a number of target cells with different activity as shown in Table III. Interestingly, IL-6 exerts a stimulatory effect (110, 111) on hepatocytes to induce the synthesis of the acute-phase proteins. More recently, the abnormal production of IL-6 and autoimmune diseases such as rheumatoid arthritis was also considered (20).

IL-6 is thought to transmit its signal through specific receptors. IL-6 receptors on cellular surfaces were studied by Taga *et al.* (112) by using ^{125}I-IL-6. There is a single class of receptors with high affinity; however, the existence of a second class of IL-6 receptors with low affinity is not excluded.

V. Concluding Remarks. It is generally accepted that the activation of resting, antigen-specific B cells into antibody-forming cells requires interaction with antigen and collaboration with T cells. Some but not all the signals delivered by intact helper T cells can

TABLE III. BIOLOGICAL FUNCTIONS OF RECOMBINANT IL-6

	Target cells	References
1. Induction of high-rate Ig secretion by activated B cells and by B-blastoid cells without growth (BCDF)	B	101, 106
2. Induction of hybridoma–plasmacytoma growth (HPGF)	B	20, 108, 109
3. Induction of IL-2 receptors on T cells	T	93
4. Induction of cytotoxic T cells (CDF)	T	20
5. Induction of acute-phase reactants in liver cells	Li	110, 111

be replaced by BSFs produced by helper T cells. Three different lymphokines, considered to be B-cell tropic, i.e., BSF-1/IL-4, TRF/BCGF II/EDF/IL-5, and BSF-2/IL-6 were identified and their cDNA were cloned (24, 25, 63, 69, 70, 95, 101). The studies with recombinant BSFs demonstrated that each BSF exerts several functions on B cells. BSF-1/IL-4 (defined as a BCGF) and TRF/IL-5 (defined as a BCDF) function similar to BCDFγ as well as BCDF and BCGFII, respectively, and BSF-2/IL-6 (defined as a BCDF) is a potent myeloma growth factor. Moreover, it was also clarified that functions of BSFs are not limited to B cells, but they show multifunctional activities on a wide variety of target cells.

It still remains unclear what the role of each BSF is in the regulation of B-cell development. For example, IL-5 has been shown to be a potent factor capable of growth and differentiation in murine B cells in a antigen-specific or polyclonal way. It is not clear, however, how IL-5 manipulates growth and differentiation of B cells on a single-cell level. In other words, it is still obscure whether IL-5 can provide both growth- and differentiation-inducing signals to each B cell at the same time, or whether each signal is effective on B cells in a certain stage of differentiation or in different stages of every cell cycle. It also remains unclear whether human IL-5 is really a growth and differentiation factor for human B cells. At this time, it is not clear whether murine IL-6 acts on murine B cells to induce terminal differentiation into Ig-secreting cells.

It is important to understand how the different interleukins interact with each other. For example, what happens to B cells when IL-4, IL-5, IL-6, and IFNγ are present together? An analysis of the controlling mechanisms of the B-cell cycle in terms of signal requirement and the establishment of an antigen-specific B-cell line will become more important in the future.

Each interleukin has diverse activity on different target cells. For instance, IL-5 acts on at least B cells, T cells, and eosinophil to induce their growth and/or differentiation. What is the prominent role of IL-5 in each cell lineage? In this respect, analysis of the regulatory mechanisms of expression of receptors for each interleukin is exclusively important. Future studies on the molecular mechanisms of the normal and aberrant regulation of the gene expression for BSFs and their receptors will provide us with information for understanding the involvement of the abnormal regulation of BSFs and their receptors in immune disorders.

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