B Cell Inducing Factor

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T cell derived soluble factor(s)

During the last two decades, great progress has been made in studies on the regulatory function of T cells in immunoglobulin production by B lymphocytes. By 1970, accumulated reports demonstrated that a cooperation between two different types of lymphocytes was essential for the full expression of humoral responses in several species (34, 47, 56). One of these cells, the T cell, is derived from the thymus, and exerts helper functions in antibody induction but does not synthesize antibody by itself, the other, the B cell, is the progenitor of antibody forming cells, B cells are functional analogues of the bursa derived cells of birds (3), and in mammals, are derived from stem cells in the bone marrow (47). With several evidences that individual precursor cells are restricted in their antigen specificity to a single antigenic determinant (12), a number of models had been proposed for the explanation of the participation of T cells in antibody production by B cells.

Based initially on studies in mice, the recognition of antigenic determinants was known to be mediated via immunoglobulin molecues on the surface of B cells. This is an essential step in humoral responses. B cells are triggered to secrete immunoglobulin when they receive the stimulatory signals through their surface receptors and a second signal from the helper molecules on, or provided by, T cells (1, 2). It was important to understand the nature of T cells that exert helper function. This function is antigen specific (34, 47) and gamma-irradiation resistant (35). McDevitt (45) and Kettman and Dutton (37) investigated the triggering of T cell activation with haptens coupled to relevant carrier proteins, elucidating a hypothesis on "educated Tcells". Due to different modes of T cell function upon antigen stimulation, the signal from T cells was proposed to be a chemical mediator (2, 5, 31). There was requirement of linked recognition by T and B cells (34, 48) that immune responses could be restored with the addition of allogeneic thymocytes to the spleen cells depleted of T cells by treatment with anti-Thy-1 serum and complement (73). In support of a possible participation of potentiating factor, Dutton *et al.* (11) reported an enhanced humoral response mediated by T cells stimulated with unrelated antigens, and Schimpl and Wecker (74) demonstrated the restored humoral responses of T cell depleted spleen cultures by a T cell product referred to as T cell replacing factor(TRF).

Soon after the first description of the non-antigen specific mediators involved in antibody responses to antigen in a T cells dependent manner, these had been confirmed by several laboratories (21, 38, 62). In attempts to understand the functional nature of T cell derived helper factors, many investigations were directed to the biochemical characterization of the factors. Using a two-chambered Marbrook system, Feldman (16) showed the antibody response without direct physical contact between T and B cells.

A ligand binding to B cell surface immunoglobulin induces a molecular redistribution on the membrane. The aggregation of antigens on the surface receptor was not sufficinent to induce antibody synthesis; antigenicity required to multivalency of the ligand (9). Parker *et al.* (53) demonstrated that anti-immunoglobulin antibodies can trigger B cell reponses. An insoluble anti-immunoglobulin reagent induced B cells to proliferate. In fact, anti-mu antibody stimulation as a mimic of

multivalent antigens was employed earlier (65).

The anti-immunoglobulin antibodies, however, did not induce antibody synthesis in murine or human B cells. Insufficient signals by anti-immunogobulin stimulation could be overcome by the helper function produced by T cells. The requirement of two signals in the induction of antibody production was first reported in rabbit B cells (41), and consequently in murine and human B cells (20, 24, 43).

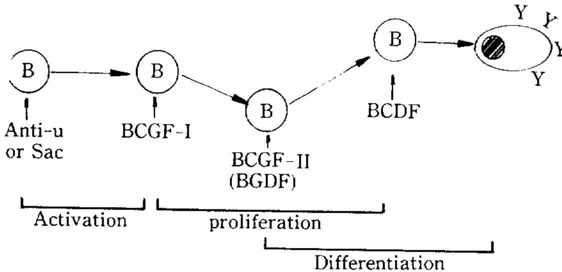

Fig. 1. Schematic depiction of the activation process of B cells and signals required for the process. (Kishimoto, *Ann. Rev. Immunol.* 3 : 133, 1985).

The T cell derived helper factors, meanwhile, were revealed to be heterogenous and to contain at least two different signals which independently exerted proliferation (B cell growth factor, BCGF) or differentiation effects (B cell differentiation factor, BCDF) on B cells (26, 49, 51, 68, 85).

To induce T cell replacing factors (TRF), three major stimulations were generally employed. T cells can be stimulated by conventional allogeneic mixed lymphocyte reactions (33, 46, 83), by mitogens (23, 81), or by *in vivo* priming followed by the relevant antigenic stimulations in vitro (22, 28, 82).

Prediction of three signals for humoral responses by Schimpl and Wecker (75) was proved with the recent progress in the studies on T cell derived immune regulatory molecules (26, 49, 51, 68, 85). Upon the discovery of non-antigen specific B cell growth factors and differentiation factors on B cells, the soluble helper factor of the T cell was resolved into a number of discrete entities. Induction of antibody production is now known to include activation of resting small B cells, subsequent proliferation of the activated precursors, and the differentiation of the activated B cells into mature immunoglobulin secreting cells.

Activation of B lymphocytes

Relatively low concentrations of anti-immunoglobulin antibodies stimulate resting B cells to enter the G_1 phase of the cell cycle (6, 7, 50). B cells can be driven to S phase by high concentrations of anti-immunoglobulin or by low concentration of anti-immunoglobulin plus B cell growth factor (BCGF) (26). Activation causes a rapid increase in cell volume, increased RNA content, and a remarkable increase in expression of class II major histocompatibility complex molecules (8). A kinetic study with various amounts of anti-immunoglobulin antibody showed that B cells in late G_1 phase only enter the S phase regardless of the presence of further anti-immunoglobulin stimulation (8, 32). The cells driven into the early G_1 phase, however, became responsive to BCGF. B cell growth factor is apparently necessary for further process into late G_1 phase of the cell cycle where macrophage derived interleukin 1 (IL-1) is required to enter into S phase (6, 15, 26).

$F(ab')_2$ fragment of anti-IgM was sufficient to trigger the activation of resting B cells. Divalency was essentially required for the activity. Anti-idiotypic antibody suppressed induction of the corresponding idiotype, which was best shown with the anti-phosphoryl choline responses by Balb/c spleen cells (4).

T cell helper function appears to be isotype specific (10, 39, 40). Production of IgG was induced with anti-IgG antibody (13). Fc-dependent feedback inhibition of this induction was also reported (54, 84). Stimulation with anti-IgA antibody is able to induce resting B cells into an activated state. Activated B cells respond to BCGF and these cells are now believed to express IL-2 receptors. But with this stimulation, there is no differentiation of B cells resulting in immunoglobulin secretion (26, 29, 39, 51, 53, 55, 85).

Staphylococcus derived protein A is a powerful agent which activates a large number of B cells, *Staphylococcus aureus* Cowan I (Sac) binds to Fab' as well as Fc fragments of antibodies (61). Immunoglobulin binding capacity of protein A was first found in 1966 (18, 66). Protein A on the matrix is able to trigger B cell proliferation on the bacterial cell (17) or on insoluble matrix such as sephadex or sepharose beads (60, 64).

Growth and differentiation and isotype expression

Since the first description of T cell derived soluble factors (11, 74), there have been extensive studies on the signals involved in antibody responses in human and murine systems. Improved modern technology made it possible to establish stable sources of T cell derived soluble factors. Even before soluble T cell factors were known, specific isotype was found in response to different antigenic stimulation. This was supported by the later experiments (52, 76, 80).

Initial murine factors that stimulate immunoglobulin secretion were characterized as molecules of 35, 000-150, 000 m.w. by gel filtration (63, 83). Kishimoto and Ishizaka reported heterogeneous factors of 20, 000-40, 000 and 150, 000 m.w. with different activities resulting in induction of different isotypes of immunoglobulin. Regulation of isotype switching is not understood at the molecular level even though genetic rearrangement of immunoglobulin genes that accompany switching have been extensively described (25, 79). T cell supernatant containing BCDF-gamma for IgG induction also contained suppressive lymphokines for IgG_2 and IgG_3. Such selective T cell effects were recently reported in murine IgA induction in Peyer's patches (36) and lacrymal glands (19), T cells secrete a variety of antigen non specific but isotype specific differentiation fractors. It is likely that the activity of different isotype inducing factors is independent of growth factors (42).

Two BCGF's from murine tumor lines are well known; BSF-p1 (26), formerly named BCGF-I, and BCGF-II (68). BSF-p1 is able to co-stimulate anti-immunoglobulin induced B cell proliferation, and this was found to have an apparent m.w. of 18, 000 (14). This factor was required during the early stage of the B cell response (27), and it increased expression of Ia antigens. Biochemical and functional characteristics of BCGF-II are well understood. BCGF-II is active only on already activated cells, but not on normal resting B cells. No co-stimulation with anti-immunoglobulin antibody alone was observed (69). It is most likely that BCGF-II has activity relatively late in the B cell response compared with BSF-p1 (67).

Meanwhile, two distinct factors with differentiative activity from three murine T cell hybridoma B151K12 (BCDF-I), and PHA induced murine molecular weights, 45, 000-60, 000 and 30, 000-40, 000, respectively, and different time of action in induction of response showed them to be discrete molecules. They were further distinguished by the synergistic induction effect they exert. The activity of the first factor (BCDF-I) was dependent on the presence of BSF-p1 and the second factor BCDF-II, while the BDCF-II factor depends on BSF-p1 and BCGF-II. The latter was clearly distinguished from IL-2.

In human, two distinct growth factors and two other differentiation factors were found. Yoshizaki *et al.* (86) reported growth factors from PHA stimulated culture supernatants and an IL-2 dependent T cell clone culture supernatant. The molecular weights on these factors were 17, 000 and 50, 000 respectively. Two differentiative factors (24, 78) revealed different molecular weights of 20, 000 and 22, 000-36, 000. These factors have very similar mode of action to those found in murine system. Both factors were different from IL-2 (24, 57, 78).

Several kinds of TRF or BCDF have reported. Some of them seem to be different from each other on the basis of the functions and the physicochemical natures, and the complexity of the factors involved in B cell differentiation

are still not revealed. Reviously in our laboratory, human T cell derived soluble factor with differentiative activity referred to as B cell inducing factor (BIF) has been extensively studied. BIF was active on Sac-activated B cells inducing immunoglobulin secreting cells. This provided a powerful model for the detailed analysis of the ISC induction in human cells (44, 57-59, 70-72).

BIF was originally induced from human T cells stimulated with PHA. DEAE cellulose separation removed the suppressive effect of the crude supernatant, which had been observed in earlier studies (62). Partially purified BIF activity was found in 20,000 m.w. fraction in gel filtration (59). This preparation also contained a considerable amount of IL-2 which co-purified in a series of chromatographic steps. BIF was purified free of IL-2 with reverse phase HPLC (59) and was active on Sac-stimulated blood B cells inducing IgM, IgG, and IgA secretion. This activity was independent from IL-2. BIF also induced increased antibody production in IgG and IgM secreting cell lines (44, 58).

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