ADVANCES IN IMMUNOLOGY

VOLUME 40

ADVANCES IN
Immunology

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VOLUME 40

1987

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
Orlando San Diego New York Austin
Boston London Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 61-17057

ISBN 0–12–022440–2 (alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

87 88 89 90 9 8 7 6 5 4 3 2 1

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Regulation of Human B Lymphocyte Activation, Proliferation, and Differentiation

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I. Introduction

The induction of an antibody response is a complex process that at a cellular level involves triggering of B cell precursors to become high-efficiency antibody-secreting plasma cells. On the basis of work by a number of investigators, a model of this process has been developed (Fig. 1) in which initial activation of resting B cells by antigen is followed by proliferation of the activated precursors and, finally, differentiation of some of the resultant daughter cells into mature nondividing antibody-secreting cells (Dutton, 1975; Schimpl *et al.*, 1974). These stages of the development of B cell precursors to antibody-secreting cells have been viewed as sequential events that are initiated and regulated by distinct and specific signals.

The basis for this model initially was derived from the experiments of Schimpl and Wecker (1972), Hunig *et al.* (1974), Dutton (1975), and Parker (1975), demonstrating that antigen or its surrogate, anti-immunoglobulin (anti-Ig), stimulated B cell activation and DNA synthesis; T cell-derived lymphokines were required to promote the subsequent differentiation of postdivisional daughter cells into antibody-secreting cells. Data of this nature suggested that antigen alone stimulated activation and clonal expansion of the B cell precursors which was then followed by terminal differentiation induced by T cell help. More recent data have suggested that regulation of B cell responsiveness may be far more complicated, with a variety of individual signals, many of T cell origin, playing a role in the activation and proliferation of B cell precursors as well as their differentiation into immunoglobulin-secreting plasma cells (reviewed by Vitetta *et al.*, 1984a; Howard *et al.*, 1984; Kishimoto, 1985).

One of the initial events involved in B cell activation is thought to be the interaction of surface Ig with antigen (Bretscher and Cohn, 1970). Depending on the nature of the ligand interacting with surface Ig and the phenotype of the responding B cell, additional signals may

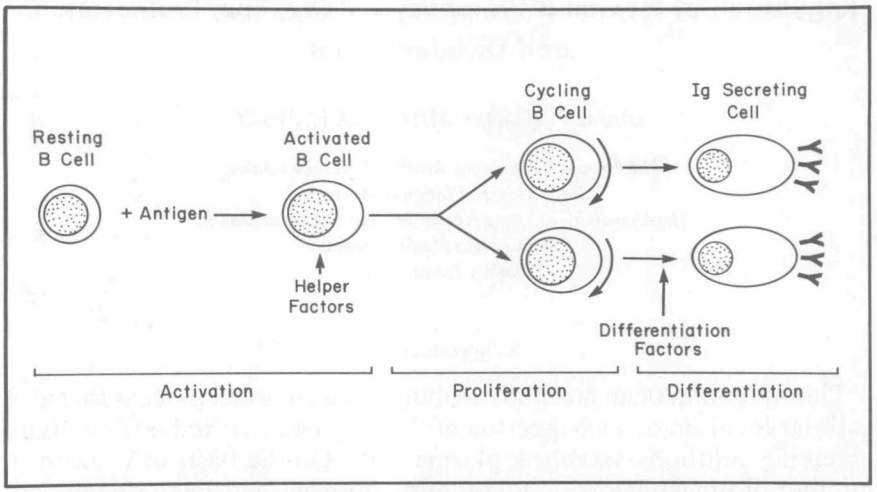

FIG. 1. Model of B cell activation, proliferation, and differentiation.

be involved in the subsequent differentiation of the activated B cell into an antibody-secreting cell. Especially important is the influence of T cells on B cell activation, proliferation, and differentiation. Although initially it was felt that only a limited number of T cell products were involved and that T cell influences were critical largely for differentiation after activation and proliferation (Dutton, 1975; Schimpl *et al.*, 1974; Schimpl and Wecker, 1972; Hunig *et al.*, 1974; Parker, 1975), more recent evidence suggests that a variety of T cell-derived lymphokines may potentially be involved in each step of B cell responses. Indeed, it appears likely that a T cell lymphokine may even play a role in preparing small resting B cells to respond before engagement of surface Ig receptors (Oliver *et al.*, 1985; Rabin *et al.*, 1985; Bowen *et al.*, 1986).

B cell responses induced by various stimuli may be discriminated by the apparent need for T cell influences to support the differentiation of antibody-forming cells. Classically, B cell responses have been defined as T cell independent (TI) and T cell dependent (TD). A number of common properties have been described that are useful in distinguishing TI antigens from TD antigens. For example, most TI antigens are poorly metabolized high-molecular-weight polymers with simple repeating structures (Britton *et al.*, 1968; Sela *et al.*, 1972). These properties have been postulated to be important in inducing cross linking of cell surface receptors, thus providing a maximal activation signal (reviewed by Mosier and Subbarao, 1982). The T

cell independence of a variety of stimuli in the murine system, e.g., polymerized flagellin (Armstrong *et al.*, 1969; Feldmann and Basten, 1971), lipopolysaccharide (LPS; Moller and Michael, 1971; Kearney and Lawton, 1975), pneumococcal polysaccharide (Howard *et al.*, 1971), haptenated or nonhaptenated *Brucella abortus* organisms (Mond *et al.*, 1974), polyacrylamide (Feldmann *et al.*, 1974), ficoll (Mosier *et al.*, 1974), dextran (Dorries *et al.*, 1974), or levan (Miranda, 1972), has been well established. Many T1 antigens such as LPS also function as polyclonal B cell activators.

TI responses have been further subdivided into two classes on the basis of the ability of TI stimuli to activate B cells from the CBA/N mouse strain which lacks the Lyb5 positive subset of mature B lymphocytes (Ahmed *et al.*, 1977). Thus, TI-1 stimuli such as LPS can stimulate B cells from CBA/N mice (Mond *et al.*, 1978a) whereas TI-2 stimuli such as ficoll and dextran do not (reviewed by Mosier *et al.*, 1977a).

Few agents have convincingly been shown to induce the differentiation of Ig-secreting cells (ISC) in man without T cell influences. Moreover, a delineation of TI-1 and TI-2 responses has not been accomplished in human studies. *Nocardia* water-soluble mitogen (NWSM; Waldmann and Broder, 1982), *Salmonella paratyphi* B (Chen *et al.*, 1981), and *Klebsiella pneumoniae* (Gross and Rucks, 1983) have been suggested to function as TI stimuli in man. It is important to note, however, that responsiveness to NWSM has been shown to be considerably enhanced by T cells (Waldmann and Broder, 1982) and inconsistent results have been obtained when responses to *Salmonella* organisms have been examined in detail (Jelinek and Lipsky, 1983). In our hands, all polyclonal B cell activators with the exception of Epstein–Barr virus (EBV) have been found to require T cells or T cell-derived factors for the generation of Ig-secreting cells in man, although some will induce B cell proliferation in a T cell-independent manner (see below). In this regard, several recent studies in the murine system have suggested that T cell influences play a role in the activation of B cells by TI antigens and thus have challenged the notion that B cell responsiveness occurs in a truly T cell-independent fashion (Endres *et al.*, 1983; Mond *et al.*, 1983; Thompson *et al.*, 1984).

TD antigens have been defined as those antigens requiring T cell help to permit the stimulation of B cell differentiation into antibody-secreting cells. T cell-dependent responses include both antigenic and mitogenic responses, each of which can be further divided into two subgroups. The first group includes responses in which physical

interactions between the B cell and T cell must take place whereas the second requires only the influence of T cell-derived lymphokines. Work in both the human and murine systems has demonstrated that small resting B cells are poorly responsive to nonspecific T cell-derived soluble factors in that such factors usually do not directly stimulate proliferation and differentiation (Andersson *et al.*, 1980; Principato *et al.*, 1983). An initial physical interaction with T cells appears to be necessary to activate such B cells, after which the cells may acquire direct responsiveness to nonspecific factors (Kuritani and Cooper, 1983; Peters and Fauci, 1983; Chiorazzi *et al.*, 1979; Peters *et al.*, 1985; Howard *et al.*, 1984; Coutinho *et al.*, 1984; Brenner *et al.*, 1984; Noelle *et al.*, 1983). By contrast, other populations of B cells may be stimulated to proliferate and differentiate in the absence of intact T cells when T cell influences are provided only by soluble lymphokines (reviewed by Vitetta *et al.*, 1984a; Howard *et al.*, 1984; Kishimoto, 1985). These results have suggested that the state of activation of the B cell subpopulation under study determines the nature of the interaction with T cells required for the initiation of T cell-dependent B cell responses. Thus, it has been suggested that "resting" B cells may require physical interactions with T cells whereas "activated" B cells may respond in the presence of soluble T cell factors. These contentions, however, have recently been challenged by several investigators. The findings of Mosier (1986) indicated that both large and small antigen-specific murine B cells required an MHC-restricted T–B cell interaction to secrete specific antibody. Moreover, in man T cell-derived lymphokines have been shown to support responsiveness of both small resting and activated B cells after appropriate stimulation (Jelinek *et al.*, 1986a). In addition, the concept that T cell-derived helper factors are not active on resting B cells has been challenged. In the murine system, Oliver and co-workers (1985) and Rabin and colleagues (1985) have shown that B cell stimulatory factor-1 (BSF-1; formerly referred to as B cell growth factor-I, or BCGF-I) stimulates small resting B cells in the absence of intact T cells. Therefore, the concept that the activation status of the B cell is the only determinant of the signals necessary for stimulation appears to be unlikely.

A variety of surface markers have also been used to delineate responsive B cell subsets (Janossy and Greaves, 1975; Ahmed and Smith, 1982, 1983). Expression of cell surface Ig isotypes has proved to be useful in this regard (reviewed by Finkelman and Lipsky, 1979; Vitetta *et al.*, 1980). Delineation of responsive subsets by virtue of cell surface IgD (sIgD) has been particularly useful. Expression of sIgD

varies during B cell maturation, activation, and differentiation. Immature resting B cells, activated mature B cells, and post-switch-memory B cells all exhibit decreased sIgD compared to mature resting B cells that express both cell surface IgD and IgM (Abney *et al.*, 1978; Sitia *et al.*, 1979; Coffman and Cohn, 1977; Black *et al.*, 1978, 1980; Preud'homme, 1977; Monroe *et al.*, 1983). Expression of sIgD by B cells can be related to the responsiveness of the population to various stimuli (Gronowicz *et al.*, 1979; Kuritani and Cooper, 1982; Saiki and Ralph, 1982; Jelinek *et al.*, 1986a). Study of B cell subsets defined on the basis of other surface Ig isotypes has also been useful in delineating functional subsets and in monitoring isotype switching during immune responses (Abney *et al.*, 1978; Kanowith-Klein *et al.*, 1981; Benner *et al.*, 1981; Teale *et al.*, 1981).

Additionally, non-Ig surface markers have also been used to delineate the functional heterogeneity of B cells. These include the Lyb5 marker in the murine system (Huber *et al.*, 1977; Ahmed *et al.*, 1977; Mond *et al.*, 1978; Mosier *et al.*, 1977b; Singer *et al.*, 1981), the B1 antigen found on human B cells (Stashenko *et al.*, 1980; Nadler *et al.*, 1981a; Boyd *et al.*, 1985a; Anderson *et al.*, 1985), and complement receptors found on both murine and human B cells (Lindsten *et al.*, 1985; Nemerow *et al.*, 1985a).

Much of what is currently understood about human B cell biology has been primarily gained through studies employing various mitogens rather than specific antigens. The use of antigen-specific B cell systems has been limited by the low precursor frequency of responding cells. However, a number of investigators have successfully studied antigen-specific human B cell responses (Hoffman, 1980a; Misiti and Waldmann, 1981; Lane *et al.*, 1981; Kehrl and Fauci, 1983; Peters and Fauci, 1983). These studies have been especially helpful in delineating the characteristics of B cells recently activated by *in vivo* immunization and have shown that these cells are largely refractory to further stimulation by antigen or polyclonal B cell activators but do respond directly to T cell-derived helper factors. However, attempts to study primary B cell responses to specific antigens have met with considerable difficulty. Therefore, an understanding of the activation of resting human B cells has largely derived from studies employing various polyclonal B cell activators.

In this article, several specific aspects of human B cell activation will be reviewed. The sequence of events and the signals involved in initial B cell activation and the relationship to subsequent proliferation and the generation of Ig-secreting cells will be delineated. The functional and phenotypic heterogeneity found among B cells as well

as activation requirements of specific B cell subpopulations will be examined. Finally, the roles of various cytokines including B cell growth factor (BCGF), interleukin 2 (IL-2), interferon-gamma (IFN-γ), and interleukin 1 (IL-1) in B cell responses and their temporal relationship to other B cell activation events will be reviewed. The goal of this review article is the development of a testable model of the generation of antibody-forming cells from human B cell precursors.

II. B Cell Proliferation and Antibody Responses

B cell proliferation plays an important role in the generation of humoral immune responses. Proliferation of B cell precursors provides a mechanism whereby a small number of antigen-specific cells can be expanded to permit the generation of an effective antibody response (Burnet, 1959). Beyond the need for clonal expansion of B cell precursors, it has been found that cell division is a necessary step in the differentiation of B cells into mature antibody-secreting cells. Thus, inhibition of B cell proliferation has been shown to prevent the generation of Ig-secreting cells in many model systems. For example, Fauci *et al.* (1978) found that pokeweed mitogen (PWM)-stimulated differentiation of anti-sheep red blood cell (SRBC)-specific antibody-forming cells from human peripheral blood B cells was inhibited by low doses of irradiation, with complete suppression of responses observed after exposure to 300 to 500 rads. Other investigators have utilized cell cycle-specific inhibitors to demonstrate the role of cell division in B cell differentiation. Jelinek and Lipsky (1983) demonstrated that treatment of human peripheral blood B cells with mitomycin C, a specific inhibitor of DNA synthesis, completely prevented the generation of Ig-secreting cells in response to PWM, Cowan I strain *Staphylococcus aureus* (SA), or *Salmonella typhimurium*. These same investigators observed similar results when hydroxyurea (HU), another inhibitor of cellular DNA synthesis (Sinclair, 1965; Krakoff *et al.*, 1968), was employed. Hydroxyurea has also been found to inhibit the generation of SRBC-specific antibody-secreting cells from murine spleen cells (Merrill and Ashman, 1980; Jaroslow and Ortiz-Ortiz, 1971).

These findings indicate that B cell proliferation plays a required role in the differentiation of antibody-secreting cells beyond the requirement to expand the number of antigen-reactive cells. Cellular division appears to play a necessary role in the maturation of precursors of antibody-secreting cells. Evidence from a variety of studies indicates that cell division is important in the differentiation of many

different lineages of cells (reviewed by Holtzer *et al.*, 1972). The precise physiologic and molecular events in the differentiation of Ig-secreting cells that depend upon cellular division remain largely unknown.

Whereas B cell proliferation appears to be necessary for the generation of antibody-forming cells from resting B cells in many model systems, the conclusion that precursor cell division is always a prerequisite for the differentiation of antibody-secreting cells remains a matter of some controversy. Andersson and Melchers (1974) have demonstrated that small resting murine B cells were able to mature to 19 S IgM-secreting cells in the absence of DNA synthesis and proliferation. In addition, Melchers *et al.* (1980) described a factor produced by antigen-activated T cells that stimulated small resting murine B cells in the presence of specific antigen to mature into IgM- and IgG-secreting cells in the absence of cellular division. A number of circumstances have also been described in which the generation of ISC in man may occur without antecedent B cell proliferation. Neckers *et al.* (1985) found that an antibody to the transferrin receptor blocked human peripheral blood B cell proliferation stimulated by SA and a T cell factor and inhibited the total amount of IgM secreted during a 5-day culture period but did not prevent IgM secretion by the cells persisting in the culture. In addition, Chen *et al.* (1981) reported that the polyclonal B cell activator, *Salmonella paratyphi* B, appeared to activate human peripheral blood B cells to produce large amounts of Ig in the absence of any detectable DNA synthesis. Similar conclusions were also reached by Gross and Rucks (1983), who stimulated B cells with *Klebsiella pneumoniae*. Finally, Grayson *et al.* (1981) reported that hydrocortisone stimulated Ig production from human peripheral blood B cells without accompanying cellular proliferation. Because B cell proliferation was only assayed by the incorporation of [^{3}H]thymidine in these studies, it is possible that division of precursor B cells occurred but the number of responding cells was so small that it could not be detected by this technique. In addition, the activation status of B cells responding to these polyclonal activators was not delineated and thus it is not known whether differentiation was stimulated from B cells that had recently divided *in vivo*. In this regard, Muraguchi *et al.* (1981) reported that an EBV-transformed human B lymphoblastoid line (CESS) responded to T cell factors with an increase in IgG-production in the absence of cellular division, suggesting that B cells at a later stage of differentiation may not require cell division before acquiring the ability to secrete Ig. It is thus possible that under certain circumstances a small population of previously acti-

vated or even small resting B cells may be induced to secrete Ig without antecedent division, although it is clear that for most precursors of Ig-secreting cells, cellular division is a prerequisite for differentiation.

In contrast to the detailed examination of the requirement of proliferation in the subsequent differentiation of antibody-secreting cells, fewer studies have addressed the role of ongoing proliferation of Ig-secreting cells in the development of the total antibody response. It has been suggested that proliferation diminishes as differentiation proceeds. Thus, for example, Ralph and Kishimoto (1981) demonstrated a negative correlation between Ig secretion and growth in phorbol myristate acetate (PMA)-stimulated human EBV-transformed B lymphocyte cell lines. Similarly, Primi (1983) found an activity in conditioned medium from a T cell hybridoma that inhibited lipopolysaccharide (LPS)-induced proliferation of murine spleen B cells but supported a high rate of Ig synthesis. These results suggested that differentiation and proliferation were mutually exclusive. By contrast, the studies of Jaroslow and Ortiz-Ortiz (1971) and Merrill and Ashman (1980) suggested that ongoing proliferation of antibody-secreting cells may be necessary for the evolution of antibody responses. These investigators found that HU inhibited the primary *in vitro* response of murine spleen cells to sheep erythrocytes when added at the beginning of culture or later when large numbers of Ig-secreting cells were

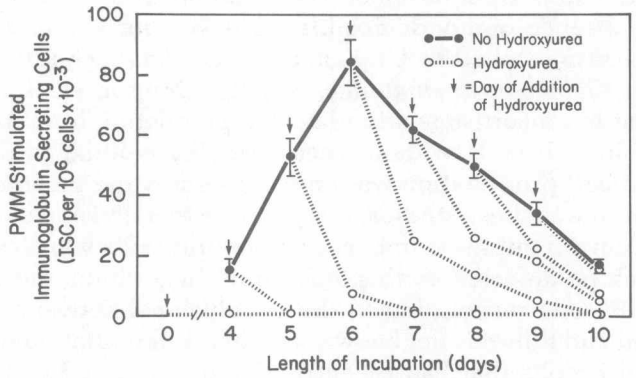

FIG. 2. Role of ongoing B cell proliferation in the continued expression of PWM-stimulated generation of Ig secreting cells (ISC). PBM were stimulated with PWM and assayed for the number of ISC found on days 4 through 10 of culture. HU (10^{-2} *M*) was added at the initiation of culture and on consecutive days thereafter. For each set of cultures, the number of ISC was then assayed on a daily basis. Control cultures consisted of PBM that were incubated in an undisturbed fashion or received saline at the same time the experimental cultures received HU. From Jelinek and Lipsky (1983).

already present. These results suggested that Ig-secreting cells were an actively dividing population but did not exclude the possibility that the antibody-forming cells were short lived and needed to be continuously replenished from a rapidly dividing precursor pool.

The possibility that B cells continue to proliferate after differentiation into Ig-secreting cells was addressed by the studies of Jelinek and Lipsky (1983). Human peripheral blood mononuclear cells were stimulated with PWM and assayed for the generation of Ig-secreting cells on a daily basis. PWM-stimulated Ig-secreting cells were detected in control cultures after a 4-day incubation, reached a maximum on day 6, and still could be detected on day 10 of culture (Fig. 2). The addition of HU at the initiation of culture completely blocked the development of Ig secreting cells throughout the length of the incubation. The addition of HU on days four through eight, when Ig secreting cells were already present, caused a marked reduction in their number within 24 hours. Similar results were noted when Ig synthesizing cells were examined by staining cells for cytoplasmic Ig. HU-resistant Ig synthesizing and secreting cells did not begin to appear in these cultures until day 6 of the incubation. The percentage of HU-resistant Ig-secreting cells increased between days 6 and 8 of culture and complete resistance to HU did not develop until day 9. To confirm that the Ig-secreting cells were actively dividing rather than being continuously replenished from a rapidly dividing precursor pool, experiments were carried out to analyze the DNA content of individual Ig-synthesizing cells. By simultaneously staining cells for DNA content with ethidium bromide and cytoplasmic Ig it was found that many PWM-stimulated Ig-synthesizing cells were cycling as evidenced by a DNA content characteristic of cells in the S, G_2, or M phases of the cell cycle (Jelinek and Lipsky, 1983). These results support the conclusion that Ig-synthesizing and -secreting cells are an actively proliferating population.

These results indicated that B cells not only require antecedent cell division to differentiate but also suggested that ongoing Ig synthesis and secretion required continued proliferation of the differentiated cells. Moreover, the expansion in the number of ISC detected as the culture was prolonged appeared to require the ISC themselves to undergo continued proliferation. These results and those obtained by other investigators in the murine system (Makela and Nossal, 1962; Perkins *et al.*, 1969; Merrill and Ashman, 1980) supported the conclusion that early in culture Ig-secreting cells are not terminally differentiated cells but rather cells capable of both continued proliferation and Ig secretion.

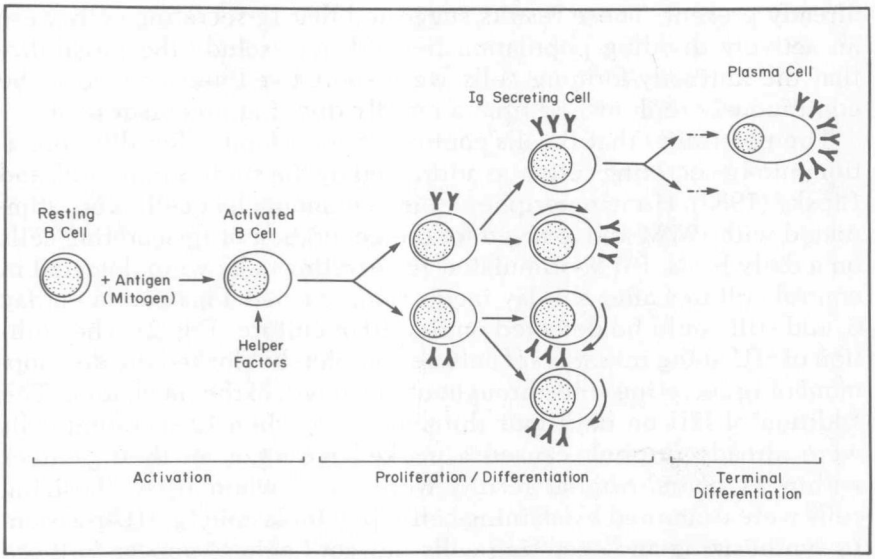

FIG. 3. Role of initial and ongoing proliferation in the differentiation of antibody secreting cells.

The model shown in Fig. 3 incorporates these results in depicting the relationship between B cell proliferation and differentiation into mature antibody-secreting cells. It is important to emphasize that the major difference between this model and the one shown in Fig. 1 is that differentiation is shown to occur during proliferation as an ongoing proliferation-requiring process rather than as a single step. The finding that early in culture Ig-secreting cells are not a stable terminally differentiated population, but rather an actively cycling one, indicates that proliferation of the Ig-secreting cells themselves plays an important role in propagating the antibody response and determining its overall magnitude. In addition, the results imply that factors that alter the continued proliferation of Ig-secreting cells after initial differentiation can affect the magnitude of the immune response. The reasons the cells undergo multiple rounds of division following acquisition of the ability to secrete Ig but before terminal differentiation into an end-stage nondividing plasma cell are not clear. However, it is known that the rate of Ig synthesis and secretion markedly increases after multiple rounds of cell division (Jelinek and Lipsky, unpublished observation). In addition, continued proliferation of the Ig-secreting cells may be important in promoting heavy chain isotype switching (reviewed by Cebra *et al.*, 1984) and providing the opportu-

nity for somatic mutations that increase the affinity of the secreted antibody (reviewed by Milstein, 1986).

Implicit in the model displayed in Fig. 1 is the assumption that the initial proliferative phase is necessary to expand the number of Ig-secreting cell precursors. To test this assumption, Jelinek and Lipsky (1985) stimulated peripheral blood B cells with SA, a polyclonal B cell activator that triggers proliferation but not differentiation into Ig-secreting cells. Addition of T cell factors supports the differentiation of Ig-secreting cells. Moreover, differentiation occurs even when cells are initially preactivated with SA alone for 48 hours and then exposed to T cell factors (Jelinek and Lipsky, 1985). Addition of HU during the initial preincubation with SA eliminated all cells traversing the G_1–S interphase and demonstrated that all the precursors of Ig-secreting cells capable of differentiating in response to SA and T cell factors resided within the population of peripheral blood B cells that were activated to proliferate by SA alone. To determine whether initial B cell proliferation expanded the precursors of Ig-secreting cells, B cells were cultured with SA alone for various lengths of time, harvested, and recultured in the presence of T cell factors (Fig. 4). When the

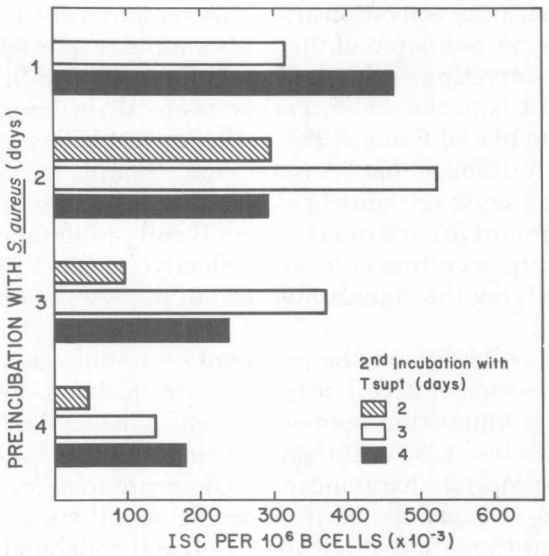

FIG. 4. Effect of preincubation with SA on the expansion of the precursors of Ig secreting cells. B cells were cultured with SA for varying lengths of time (1 to 4 days). Cells were then washed and recultured in the presence of T cell supernatant. Cells were assayed for the generation of Ig secreting cells after 2 to 4 days of the second incubation. From Jelinek and Lipsky (1985).

number of Ig-secreting cells generated was examined after the second incubation, it was found that the precursor pool of Ig-secreting cell progenitors did not appear to be preferentially expanded as the SA-stimulated culture was prolonged beyond 24 hours. This observation indicated that the precursors of Ig-secreting cells are not selectively expanded as a result of SA-induced proliferation and therefore supported the conclusion that in this model system proliferation is necessary to induce responsiveness to differentiative influences but not to increase the size of the responsive precursor pool.

III. Induction of B Cell Responses

Much of our current understanding of human B cell responsiveness has derived from studies using T cell-dependent polyclonal B cell activators (PBA). These include PWM (Janossy and Greaves, 1975; Keightley *et al.*, 1976) and soluble staphylococcal protein A (SPA) (Sjoquist *et al.*, 1972; Lipsky, 1980). Both B cell proliferation and the generation of antibody-secreting cells stimulated by these PBA require T cell help. In addition, anti-immunoglobulin antibodies (anti-Ig) and SA are also T cell dependent but represent a different class of stimuli in that initial activation and DNA synthesis are T cell independent whereas perpetuation of the proliferative response and the generation of Ig-secreting cells are T cell dependent (Sell *et al.*, 1965; Kishimoto and Ishizaka, 1975; Parker *et al.*, 1979; Romagnani *et al.*, 1981; Muraguchi and Fauci, 1982; Jelinek and Lipsky, 1985). In man, anti-Ig and SA differ in that SA is a potent stimulus for the generation of Ig-secreting cells but anti-Ig is not. Since soluble T cell-derived factors can support SA-activated human B cell proliferation and differentiation into Ig-secreting cells very effectively, this PBA has proved useful for studying the signals involved in the regulation of human B cell responses.

Although T cell help may be provided by soluble factors in a number of systems, some B cell responses are initiated only through a direct physical interaction between T cells and B cells (reviewed by Singer and Hodes, 1983). Antigen-specific or linked recognition requires the appropriate hapten-carrier conjugate to stimulate the hapten-specific B cell and the carrier-specific T cell simultaneously. At one time it was thought that effective T–B cell collaboration involved a direct physical interaction between the T cell and the B cell via an antigen bridge (Mitchison, 1971; reviewed by Singer and Hodes, 1983). More recent studies, however, have documented the ability of B cells to serve as antigen-presenting cells (Chestnut and Grey, 1981;