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THE UNIVERSITY OF ALBERTA

ROLE OF INTERLEUKIN-2 AND INTERLEUKIN-2 RECEPTOR IN B CELL PROLIFERATION

by

LUCINE O. BOSNOYAN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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To My Parents

Abstract

Resting human B cells can be activated to proliferate in the presence of both polyclonal antibodies to immunoglobulin mu heavy chains and B cell growth factor (BCGF). The role of IL-2 in B cell proliferation and expression of activation antigens such as IL-2 receptor, transferrin receptor, HLA-DR, 50H19, 4F2 was investigated. There is a synergistic effect between BCGF and IL-2 on B cell proliferation which is concomitant with an increase in activation antigen expression. This response was inhibited by antibody to the IL-2 receptor. Furthermore, antibodies to some but not all activation antigens also inhibited this response.

These results demonstrate that IL-2 acts directly on B cells through IL-2 receptors expressed on them. However, in contrast to T cell proliferation, BCGF is needed to trigger activation of the IL-2 receptor gene since IL-2 alone does not trigger proliferation of B cells.

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My special thanks go to my parents for their love.

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BCGF:	B cell growth factor
BSF:	B cell stimulatory factor 1
BCDF:	B cell differentiation factor
IL-2:	Interleukin-2
IL-2R:	Interleukin-2 receptor
ISC:	Immunoglobulin secreting cell
TRF:	T cell replacing factor
HLA:	Human leukocyte antigen
FCS:	Fetal calf serum
Ca^{++}:	Calcium
PHA:	Phytohemagglutinin
PI:	Propidium iodide
FITC:	Fluorescein isothiocyante
IL-1:	Interleukin-1
LPS:	Lipopolysaccharide
PMA:	Phorbol-12-myristate-13-acetate
KLH:	Keyhole limpet hemocyanin
mRNA:	Messenger ribonucleic acid
cDNA:	Copy deoxynucleic acid
Na^{++}:	Sodium
SRBC:	Sheep red blood cells
PBL:	Peripheral blood leucocytes
PBS:	Phosphate buffer solution

INTRODUCTION

In the last few years much research has been carried out to understand the most fundamental of biological processes, i.e., unravelling the molecular basis of the control of cell division. That is to understand the biochemical nature of the molecules which initiate and control cell growth and differentiation, and to identify and clone the genes encoding them. Concurrently, hybridoma and genetic engineering technology has become available for the large scale production of the cytokines responsible for stimulating cell growth and differentiation. This has allowed not only their physicochemical characterization, e.g., molecular weight and protein sequence, but also for preparation of large amounts of the purified factors for performing biological experiments to ascertain the precise function of these molecules in the concatenation of molecular events that results in cell proliferation and differentiation. At this stage, I wish to define cells which are in the "proliferating" state, as those that traverse the cell cycle and pass through the familiar events of DNA replication and mitosis, and at the same time

are increasing their mass through production of the new proteins and other components that are needed to produce a new cell. Non-proliferating cells can be in a quiescent steady state, G_0, in which they are metabolically active but do not proliferate or show no synthesis of molecules. Growth is dependent on a switch from one of these states to the other and this switch is dependent on external factors, e.g. nutrients, growth factors, and hormones. As by the definition by Pardee et al., (1985), the G_0-S. interval should be divided into two main periods. A variety of experiments involving oncogenes, growth factors, nutrients, and inhibitors show that the events in these two intervals quite different. In the first half the cells emerge from quiescence through a competence and progression process, and synthesize new mRNAs and proteins. In this interval from G_0 to mid G_1 point the cell restores its machinery for making mRNAs and proteins. Then, in the second half of the G_0-S period the cell prepares for DNA synthesis.

Hematopoietic and lymphoid cells provide useful experimental systems to ascertain the cascade of molecular events from triggering to processing of signals since some markers exist on for monitoring the acquisition of specific functions that occur during differentiation like

acquisition of specific receptors (IL-2 receptor, T cell receptors) or synthesis of proteins linked to a specific function of the differentiated cells (immunoglobulins, hemoglobin, and peroxidases etc.).

B lymphocyte has recently provided a good model of such a differentiated system, in which antigen-activated, lymphokine-stimulated proliferating B cells are acted upon by another group of lymphokines named T cell-replacing factors: B cell growth factor (BCGF, or BSF1), B cell differentiating factors: (BCDF or BSF2 or BCGF II) which together cause the immature B cells to proliferate and to become immunoglobulin producing plasma cells. These factors are so far B cell restricted. But in fact the situation is more complex than it has been shown, that other lymphokines can play a role on B cell proliferation and differentiation (gamma interferon γIF, IL-1, IL-2).

T lymphocytes also provide, a good model of cell activation by antigen(s) in which a series of lymphokines (IL-2, IL-1) will play a role in proliferation and differentiation. However there is more evidence that IL-2 is not restricted to T cell alone. Recently reports have shown evidence of its receptor's expression on B cells, and the role it plays in B cell differentiation.

Thus by choosing B lymphocyte activation as a model I wanted to look at the role IL-2 could play in B cell proliferation in association with BCGF. Further questions related to the mechanism of action of B cell growth factor (BCGF) in B cell proliferation, remain open for discussion. For example, is the binding of BCGF to its receptor the only signal required for B cell proliferation? Alternatively, does the binding of this lymphokine activate the genes for receptors of a variety of other growth factors (such as Interleukin 2 IL-2) which may play an important role in monitoring the processing of BCGF signal. These receptors may be equivalent to some, but not all, of the "activation markers" that will be described in this chapter. Furthermore, does BCGF induce a cascade of transduction systems required for the processing of the signals involved in cellular proliferation and/or differentiation? Will some of the activation markers found on proliferating B cells turn out to be the structural elements of such systems which may be common to all proliferating cell types.

I. T CELL: GENERAL MODEL FOR CELL ACTIVATION, PROLIFERATION AND DIFFERENTIATION.

Several lines of evidence have led to the conclusion that T cell mitosis results from the interaction of soluble T cell-derived factors with antigen/lectin stimulation. It is important conceptually at this point, to discard the term "factor" because a factor promotes no connotation as to a mechanism of action. The word "hormone" carries with it, however, criteria that have immediate physiological implications. Hormones demonstrate strict target cell specificity, and exert their effects at low concentrations. They are released in response to environmental variations and are under a feedback regulatory control in most instances. Moreover, once it is understood that some lymphokines interact with target cells through specific receptors it immediately implies that postreceptor mechanisms are operative to mediate the biological effect.

One such excellent example is IL-2, formerly called T cell growth factor, which provides a necessary signal for the transition of activated T cells from the G_1 to S phase of the cell cycle. It appears however, that IL-2 receptor expression does not appear to be confined to cells of the T cell lineage but can also be induced on normal B lymphocytes.

A. ROLE OF IL-2 RECEPTORS IN T CELL GROWTH AND DIFFERENTIATION.

Interleukin-2 was first described as an activity that initiated T-cell mitosis after antigen or lectin activation, Morgan et al., (1976). Recently a monoclonal antibody (anti-Tac and others) against the IL-2 receptor has made it possible to begin to understand the importance of the T-lymphocytotrophic hormone, IL-2.

In order to exert its biological effects, IL-2 must interact with specific high affinity membrane receptors. This interaction is critical to the evolution of a normal immune response. IL-2 receptors are not expressed in resting T cells, but they are expressed rapidly following activation with antigen or mitogen. IL-2 receptor has been identified as an Mw=55000 glycoprotein on normal activated peripheral blood T cells. These receptors are composed of a peptide precursor of Mw=33000(p33). This precursor is cotranslationally modified to a p35/p37 doublet by N-linked carbohydrate addition, and then modified in the Golgi apparatus to the Mw=55000 form. Mature receptors contain N-linked and O-linked carbohydrates and sialic acid, and are both sulphated and phosphorylated. By using the monoclonal antibody anti-Tac and specific inhibitors, of protein or DNA synthesis in Con A-stimulated cultures, Uchiyama, et al., (1981 a, b).

Leonard, et al., (1982) (1983). have found that the expression of Tac antigen requires protein synthesis but not DNA synthesis or cell division, and that the IL-2 receptor interaction is obligatory; Monoclonal antibodies reactive with IL-2, or the IL-2 receptor, inhibit T cell proliferation by preventing IL-2 receptor binding in a concentration-dependent manner. Moreover, anti-Tac suppresses IL-2-dependent T-cell proliferation at concentrations remarkably coincident with those that block IL-2 receptor binding. These observations suggest strongly that anti-Tac reacts with the IL-2 receptor, although it remains unclear whether the epitope recognized is part of the IL-2 binding site: IL-2 does not compete for anti-Tac binding even when used in a 100-fold molar excess over that required to saturate IL-2 binding Smith, (1984).

In the case of T cells IL-2 alone can upregulate IL-2 receptor expression. Depper et al., (1985) have shown the same results on PHA stimulated T cells while Malek and Ashwell (1985) reproduced the same results on a T-cell clone. IL-2 receptor upregulation was maximal after 12 h and required new RNA and protein synthesis. RNA blots hybridized with an IL-2 receptor mRNA accumulation after incubation in purified IL-2. Transcription assays performed with isolated nuclei further demonstrated that, at least in part, the increase in IL-2 receptor mRNA

resulted from IL-2 activation of IL-2 receptor gene transcription. However, IL-2 alone does not induce IL-2 receptor expression in freshly isolated lymphocytes, it appears to be required for optimal receptor expression when such cells are activated with PHA or anti-T_3. Thus PHA stimulated cells require two or more signals in combination in order to express IL-2 receptors. The reconstitution of IL-2 responsiveness in these cells with small concentrations of PHA suggests that the continued presence of a competence signal, whether mitogen or antigen, may be required for IL-2 receptor upregulation by IL-2. This synergy of IL-2 and a second signal is further demonstrated by large increases in IL-2 receptor mRNA accumulation after stimulation with both IL-2 and submitogenic concentrations of PHA.

Recent evidence showed that some T cell activation antigens might play a role in IL-2 receptor expression. Reed et al., (1985) and Nowell et al., (1985) have reported that antibodies recognizing the 50Kd protein associated with the sheep erythrocyte receptors namely OKT11A, 9.6, and 35.1, will suppress lectin mitogen-induced T cell proliferation by impairing both IL-2 elaboration and IL-2 receptor acquisition. Other recent studies have demonstrated that the 50 Kd T11 protein is a surface component of a macrophage-independent alternative pathway

of human T-cell activation is unrelated to the T_3/T_1 antigen-MHC receptor complex. The triggering of T11 by monoclonal antibodies anti-$T11_2$ and anti-$T11_3$, directed at two unique epitopes on the molecule, induced IL-2 receptor expression on both $T3^+$ and $T3^-$ thymocytes did not proliferate in response to anti-$T11_2$ and anti-$T11_3$ in the absence of exogenous IL-2. Those studies by Fox et al., (1985) suggested that IL-2 receptor gene activation precedes IL-2 gene activation in T-cell development.

IL-2-mediated effects were not confined solely to induction of its own receptor. Both c-myc and transferrin receptor mRNA levels were increased after stimulation with IL-2 suggesting that IL-2 initiates a program of transcription of other genes involved in lymphocyte activation. Consistent with this, the increase in transferrin receptor mRNA temporarily occurred after the increase in IL-2 receptor mRNA. In contrast to these antigens selectively expressed during cell activation and growth, IL-2 did not induce increases in the expression of mRNA for the β chain of the T-cell antigen receptor, Depper et al., (1985). Thus, IL-2 is required for optimal IL-2 receptor expression during the course of lymphocyte activation in the presence of lectin or antigen, and it can augment transcription of the IL-2 receptor gene in cultured cells maintained in a competent state of activation.

However, IL-2 alone is insufficient to maintain a continuous state of maximal transcription of the IL-2 receptor gene.

Additional evidence of autoregulation of IL-2 was recently published by Smith and Cantrell (1985) whereby they have shown that IL-2 binding sites and anti-Tac reactive epitopes differ markedly in their respective turnover characteristics; moreover, IL-2 actually regulates the expression of IL-2 receptor molecules, and consequently IL-2 governs the magnitude of its own biologic response by a unique mechanism, manifested by an IL-2 promoted diminished expression of IL-2 binding sites, and an increased expression of IL-2 receptors that are recognized by anti-Tac but do not bind IL-2 with high affinity. Information regarding the mechanism by which the ligand-receptor interaction promotes a switch in receptor expression should be forthcoming from investigations of receptor gene expression. Recent studies done by Nikaido, et al., (1984), and Leonard, et al., (1984) have shown that there are two IL-2 receptor mRNA transcripts suggesting it arises from one gene. Accordingly Smith and Cantrell interpreted their findings to show that only one of the two transcripts translates molecules containing a high-affinity IL-2 binding site, whereas both transcripts encode receptor molecules that express the Tac epitope.

However, recently Leonard et al., (1985) have predicted from the structure of IL-2 receptor gene that as many as 18 different mature mRNA forms can be produced by the single gene for the IL-2 receptor sequenced through the analysis for three different transcription initiation sites for alternate mRNA splicing and at least three different polyadenylation signals on the gene they have sequenced.

Another interpretation of the high and low affinity cellular receptors for IL-2 by Robb, et al., (1984) was that it might be due to the molecular heterogeneity of the Tac protein, which is probably generated by differences in posttranslational modifications which include sulfation, disulfide bonding, phosphorylation, and extensive glycosylation. In addition , the variation in IL-2 receptor affinity could be due to conformational changes caused by interaction of the Tac protein with a different hypothetical receptor subunit, Greene, and Robb, (1985) or by dimerization of the Tac molecule so that the active site of IL-2 receptor remains to be identified. Further investigation is required to define whether the receptor exists as an isolated surface glycoprotein or alternatively a receptor complex. Some aspects of those questions could be answered through the last investigation by Leonard et al., (1985), whereby they have sequenced the gene encoding the human IL-2 receptor it consists of 8

exons spanning more than 25 kilobases on chromosome 10. Exons 2 and 4 were derived from a gene duplication event and unexpectedly also are homologous to the recognition domain of human complement factor B. The stoichiometry and specific site or sites of IL-2 binding to IL-2 receptors are unknown, but the internal gene duplication raises the interesting possibility that the duplicated domains may be critical to IL-2 binding and that there may be two IL-2 binding sites per receptor molecule.

II. B CELL ACTIVATION, PROLIFERATION AND DIFFERENTIATION.

The discovery that B lymphocytes bear Ig on their surface, Sell and Gell (1965) led to the formulation of two divergent theories about proposed roles for surface immunoglobulins (sIgs) on B cell activation. In a one-signal model, Coutinho and Moller (1974) postulated that the major role of sIg on the B cell was to focus antigen onto the surface of that cell. Once focused on the cell, the mitogenic portion of some antigens (thymus independent TI antigens) would deliver a proliferative signal to the cell and induce clonal expansion and antibody secretion.

Those antigens lacking a mitogenic portion (thymus-dependent TD antigens) would focus T cells onto the surface of B cells where T cell derived signals could induce B cells to grow and differentiate. In contrast to this theory, in a two-signal model, Bretcher and Cohn (1970) suggested that antigen mediated cross linking of sIg itself provided the first signal in the initiation of B cell activation. However, this signal per se was insufficient to induce B cells to differentiate into antibody secreting cells. A second signal requiring the participation of T cells and their soluble products, was necessary, so that different triggering signals are required for the generation of the cascade of activation events. Since both the differential gene expression, as well as the metabolic activity are under the influence of signals from the extracellular surrounding, their effects do not directly act on the intracellular target, rather their effects are processed through different elaborate signal systems. These are:

a. specific receptor proteins,

b. proteins involved signal transformation in many instances secondary signal (secondary messengers) formed within the cell,

c. regulatory protein interacting with parts of the gene expression chain.

The whole sequence of signal processing provides the means for amplification and for transport of the information to the sites of intracellular action as well as for degradation of the primary and secondary signals.

One system for the study of signal processing is in the B cell where one signal is needed for activation and a second for proliferation and a third for differentiation.

A. B Cell Activation.

B-cell activation is a complex mechanism, whereby early events of crosslinking of surface Ig receptors by anti-Ig or antigen is followed by a decrease in membrane potential, influx of Ca^{2+}, hyperexpression of Class II antigens, (Monroe and Cambier, 1983; Mond et al., 1981; Monroe and Kess, 1985; Monroe et al., 1984), cell enlargement, and entry into the G_1 phase of the cell cycle. Among these events is the expression of activation antigens which appear only during activation and are absent on resting B cells.

Different stages of cell activation may serve as control points and provide greater sensitivity for regulation of B-Cell responses. Furthermore, the ability to perceive various signals at different stages of activation may allow greater flexibility in regulating response patterns, Wetzel et al., (1984). Hence,

identification of different activation states may provide insight into biochemical and genetic processes involved in activation and regulation of B-cell responses.

The concept that both antigenic and mitogenic activation of resting B cells results in functional expression of receptors for soluble proliferation and/or differentiation cofactors was initially proposed by Andersson and Melchers (1981) and Andersson et al., (1980). In the subsequent discussion I will emphasize the studies performed to attempt to demonstrate the existence of antigen non-specific factors which transmit signals for the growth and/or differentiation of human B-lymphocytes. In the models proposed by Kehrl et al., (1984), the initial signal is provided by the interaction of antigen or its equivalents with the surface membrane Ig. This initiates B cell activation, and the cell expresses receptors for growth factors. In the presence of appropriate growth factors, the cell enters S phase and becomes a cycling cell. Subsequently receptors for differentiation factors are expressed, and the presence of those factors initiates the production and secretion of Ig.

Surface Ig is an unusual receptor since not only can it bind its ligand but, in another form, it is secreted as a functional antibody. Membrane Ig differs from secreted Ig in its COOH-terminal region where it has a hydrophobic tail

which anchors it to the membrane. Apparently only three hydrophilic amino acids of membrane Ig are intracytoplasmic, and these are not phosphorylated, Kehrl, et al., (1980). How surface Ig after it binds antigen can transmit a signal for proliferation with such a small cytoplasmic tail, is not known. It has been suggested that capping of surface Ig may be necessary for triggering, Braun and Unanue (1980). During this process, surface Ig may need to interact with other membrane structures capable of serving as "bridges" into the cytoplasm to signal proliferation. A particularly valuable system for addressing events related to B-cell activation has been the use of affinity purified anti-Ig antibodies as polyclonal analogs for antigen, first developed in rabbit by Sell and Gell (1965) and adopted to mouse lymphocytes by Parker (1975) and Weiner et al., (1976). Anti-Ig stimulation combines the advantages that it acts on the receptors for antigen for virtually all B cells and that the resultant activation is restricted to DNA synthesis without development of antibody forming cells, Kishimoto and Ishizaka (1975), Parker et al., (1979).

Parker et al., (1980, 1982) and Muraguchi et al., (1984)., using anti-Ig stimulation of B cell provided evidence that soluble T cell derived factors, which had no apparent effect on resting B cells, enhanced

anti-Ig-induced B-cell proliferation particularly when relatively low concentrations of anti-Ig were used. (5-10ug/ml). He also demonstrated that although the concentrations of soluble anti-Ig which activate B cells are the same as those which induce capping and endocytosis of receptor Ig, the response to anti-Ig beads suggested that activation is entirely a surface event, and that internalization of receptor Ig is not required for proliferation or differentiation to Ig secretion. Also in the same system as mentioned above he provided evidence that cross-linkage of surface Ig is the inductive signal to Ig secretion, as well as for the proliferative response to anti-Ig, since the bivalent fragment $F(ab')_2$, fragment of anti-Fab antibody induces a response while the monovalent Fab' fragment does not. Further reviews on the role of Ig receptor involvment in signal transmission to the cell were done by Schimpl, (1984) and Tony, and Schimpl, (1980). Still the explanation for the different effects of anti-u at low versus high concentrations is unknown. One suggestion by Kehrl et al., (1984b) is that by choosing a low concentration of anti-u, a particularly useful system could be developed for dissecting the minimal and optimal signals required for the induction of resting B cells from G_0 to G_1 (blast transformation) and G_1 to S phase. Similarly DeFranco et al., (1982) showed that low and high

concentrations of anti-u produced identical effects during the first 24 h of culture, while subsequent progression of B cells into S phase was dependent upon high concentrations of anti-u being present during the remainder of the culture, their explanation for the difference of response being due to different B cell subsets having distinct activation requirement. In a recent publication DeFranco et al., (1985) have further dissected their model of different requirement for anti-IgM concentration in the early phase of G_o into G_1 where low concentrations 1-to 5 ug/ml of anti-IgM are required and at late G_1 fivefold to 50-fold higher concentration of anti-IgM are required. Thus B cells may exist in states of partial activation and must possess a mechanism to integrate the amount of stimulatory signals they have received; they enter a commitment period for S phase only when that signal passes some threshold value. These results suggested that there is a control point for progression into S phase 24 to 36 h after stimulation and transition through that control point may be related to growth factors. Thus supporting the view that T cell signals were necessary to drive activated B cells to proliferate, (Howard and Paul 1983 and Paul 1985).

Darzynkiewicz et al., (1980) have divided the G_1 phase of the cell cycle into two subcompartments, G_{1A} and G_{1B}. An increase in RNA content above a critical level

was required for the transition from G_{1A} to G_{1B}. Exit from G_{1B} and further progression into S phase also appeared to correlate with cellular RNA content. In this context, low concentrations of anti-u may be suffitient for the G_O to G_{1A} transition, while an additional signal such as BCGF is needed for G_{1A} to G_{1B} transition. After attainment of G_{1B} state the activation cells continue to synthesize RNA and progress into S phase.

The second common anti-u reagent used is Staphylococcus aureus Cowan strain I (SAC). As SAC binds to the Fab as well as the Fc portion of human Ig, SAC can bind to and crosslink surface Ig with a resultant powerful activation signal Romagnani et al., (1981).

The signal provided by SAC not only delivered the initial activation signal but also provided sufficient stimulus for the progression of B cells into S phase on the apparent absence of exogenous growth factors. This conclusion was based upon limiting dilution studies performed by Falkoff et al., (1983) using highly purified B cells stimulated with SAC. However, T cell help was absolutely necessary for the differentiation of B cells into Ig secreting cells (IgG), Falkoff et al., (1982). A study of the sequence of events involved in the activation and proliferation of B cells and the relationship of activation and proliferation to the subsequent

differentiation of B cells into ISC was further reviewed by Jellinek and Lipsky (1985).

1. ACTIVATION MARKERS IN THE CELL CYCLE PROGRESSION OF HUMAN B CELLS

a. Activation Antigens: The Role They Could Play in Processing the Signal for B-cell Proliferation.

A recent publication by Boyd, et al., (1985a) shows the cell surface phenotype variation associated with the different stages of B cell development i.e., activation, proliferation and differentiation. By using a panel of monoclonal antibodies defining B cell surface antigens four groups of antigens were identified by their expression during the stages of anti-Ig-induced activation and differentiation. The first was a pan-B cell group: B1, B4 and Ia were all present on resting B cells, but increased after activation from day 1 to 5 and declined during differentiation. The second group consisted of a single antigen, B2, which was rapidly lost after activation. This places B2 in a peculiar position indicating that B2 may be intimately involved in the events of activation. The third group consisted of five antigens (B5, BB1, B-LAST$_1$, T 12 and IL-2R). None were expressed on resting cells and all appeared at 24 to 48 h post-activation. The levels of each peaked at 3 to 4 days

but declined during the differentiative phase. The fourth group comprised the differentiation antigens, T10, PCA-1, and PC-1.

In attempting to classify activation antigens and the role they could play in the activation process Clark et al., (1985) have demonstrated the role of 35KDa polypeptide, Bp35 first defined by the MAb B1 expressed on all B cells, plays in B-cell activation. MAb to Bp35, alone or with T-cell derived factors (MLR-TF) induced B cells to divide. This was the first evidence to show that human B cells can be induced to proliferate via a surface polypeptide distinct from surface Ig. Although not formally demonstrated to be such a bridge, Clark et al., (1985) suggested that Bp35 molecule does meet a number of the requirements that would be necessary for one: it is on the surface of all B cells at high density; when it is crosslinked directly, B-cell proliferation is induced; and it is a phosphoprotein, which would be necessary if a phosphokinase-dependent pathway is utilized.

Another candidate for B-cell activation marker is B5, described by Freedman et al., (1985). The functional role of B5 in the process of activation is still unknown except that its appearance on the B cell surface after activation, suggests that it is one of the earliest B-cell activation antigens. In contradiction to Bp35, described previously,

its likely candidates for receptors for growth and differentiation is questionable since anti-B5 cannot trigger B cells to proliferate, nor can it block proliferation of anti-Ig-stimulated B cells by BCGF or IL-2.

Recently, more is investigated on the role B1 molecule could play in the regulation of B-cell activation and differentiation. B1 is a B lineage-restricted molecule, described by Stashenko et al., (1980). B cells activated by crosslinking of sIg begin to express B1 and HLA-DR molecules at higher densities, Boyd et al., (1985). Tedder et al., (1985) have demonstrated that anti-B1 antibodies significantly inhibited B-cell proliferation induced by anti-u antibodies, SAC activated T cells and EBV virus. Although capable of inhibiting proliferation, anti-B1 antibody, in soluble form or coupled to beads, did not activate B cells or induce proliferation. Comparable inhibition of B-cell activation was noted with antibodies reactive with Class II antigens of the major histocompatibility complex with the exception that anti-B1 antibody inhibited immunoglobulin secretion in pokeweed mitogen assays, whereas anti-DR antibody did not.

Haynes et al., (1981) described the monoclonal antibody (MAb)4F2 which recognizes an antigen present on activated lymphocytes but absent from resting lymphocytes. 4F2

recognized a non-HLA, non-Ia cell surface determinant which is a disulfide glycoprotein complex (125,000 dalton) composed of a sialylated heavy subunit and an unsialylated light subunit (Hemler et al., 1982). Kehrl et al., (1983) have studied the expression of the 4F2 and 5E9-defined glycoproteins on in vitro-activated B cells in relation to the cell cycle. Neither glycoprotein was present on resting B lymphocytes; however, following in vitro stimulation with anti-u, both glycoproteins were expressed. The expression of 4F2 correlated with blast transformation (G_o to early G_1 transition), while blast transformation alone was insufficient for 5E9 expression described by Haynes et al., (1981) as the transferrin receptor. 5E9 was expressed later in the B cell cycle, and the presence of 5E9 correlated with the early to late G_1 transition. All cells which progressed into S, G_2, and M phase were both 4F2- and 5E9-positive.

This glycoprotein undergoes variable glycosylation to form heavy subunits of Mw 93000 (B cell lines) and Mw 85000 (T cell lines). Hemler and Strominger (1982) reported that this represents the first such example of a glycosylation difference between T and B cell antigens whose polypeptides are the same size and presumably identical in amino acid sequence.

The functional significance of the glycoprotein defined by the monoclonal antibody 4F2 on human B and T lymphocytes and other cell types is unknown at present. Kehrl et al. (1984 a,c,) provided evidence that 4F2 may be an important lymphocyte surface molecule, by the fact that 4F2 monoclonal antibody addition to purified B cells which were immunized to either pneumococcal polysaccharides or with soluble protein antigen keyhole limpet hemocyanin (KLH) could significantly suppress the specific spontaneous antibody production.

Meanwhile, the addition of 4F2 MAb to pokeweed mitogen-stimulated cultures of peripheral blood mononuclear cells greatly augments antibody production. This observation was shown to be mediated by an effect of 4F2 on T cells. The presence of 4F2 in culture partially suppressed DNA synthesis by B and T cells in mitogen-stimulated cultures. These findings suggest that 4F2 recognizes a cell surface molecule that is important in human lymphocyte responses.

B. GROWTH FACTORS AND THE ROLE THEY PLAY IN B CELL GROWTH AND DIFFERENTIATION

1a. Transferrin Receptors

Transferrin receptors are expressed on all proliferating cells *in vivo* and *in vitro* and appear to be essential for cell growth. Transferrin receptor expression enables cells to import transferrin, the predominant serum iron-binding glycoprotein. Transferrin-mediated delivery of iron is absolutely required for eukaryotic cell growth.

Transferrin receptors can be detected after but not before mitogenic stimulation of normal peripheral blood T and B cells. This expression is not only a phenotypic characteristic of activated T cells but a functional requirement of the activation process as well, and furthermore, appears to be regulated by T-cell-specific growth factor IL-2. Thus far little is known about these genes that control cell growth and differentiation relate to each other. It has been demonstrated by Neckers et al., (1983) that an interaction of IL-2 with its receptor is required for the expression of transferrin receptors. Kronke et al., (1985) in their efforts to further understand the regulation of the genes encoding c-myc, IL-2, IFN-γ and receptors for IL-2 and transferrin, have studied the kinetics of induction of these genes during T lymphocyte activation. Using nuclear transcription assays they have demonstrated that expression of each of these genes is regulated at the level of transcription induced independently of de novo protein synthesis with the

exception of the transferrin receptor gene transcription which is initiated late in the course of cell activation and the expression of which required de novo protein synthesis.

Considerable interest and work has been focused on the identification of cell surface antigens involved in the regulation of cell activation with the goal to use specific agents including antigen, lectins, tumor promoters, and mAbs to monitor and alter cell function in vivo. Each of these activation signals ultimately influence cell growth and differentiation either by altering the properties or rates of synthesis of existing proteins or by initiating the synthesis of new ones. Included among the newly induced network of cellular proteins are the IL-2 receptor, transferrin receptor, and the lymphokines, IL-2 and interferon (IFN-γ) Kronke et al., (1985).

1b Regulation of B-Cell Proliferation by Transferrin Receptor.

Regulation of B cell proliferation is not well understood, and for many years has been an area of great controversy. It remains unclear whether B cell proliferation is required for differentiation to occur or whether the two events are independent of each other. Neckers et al., (1984) showed that BCGF interaction with its receptor results in transferrin receptor expression and

proliferation. By using anti-transferrin monoclonal antibodies, they were still able to show secretion of immunoglobulin indicating that proliferation may not be required for differentiation. These findings support a model of lymphocyte activation in which proliferation is dependent on induction of transferrin-receptor in the late G_1 phase of the cell cycle. Transferrin receptor induction is regulated by cell-specific growth factors (IL-2, BCGF) whose own synthesis is tightly controlled. This model, which can apply to other tissues as well, implicates transferrin, a ubiquitous serum glycoprotein, as a major growth regulator of many cell types. Cells regulate their sensitivity to transferrin by regulating the level of transferrin receptors on their surface. Most B cell lymphomas do not possess IL-2 receptors, nor do they respond to IL-2 yet they still require transferrin receptors for growth. Transferrin plays an essential role in the maintenance of growth of continuous cell lines, and is an essential component of serum-free medium. Blockade of surface receptors for transferrin inhibits the growth of many cell lines, also DNA synthesis in mitogen-stimulated lymphocytes. The mechanism by which transferrin exerts its effects on growing cells is not known. Because the concentration of transferrin in serum is quite high (4 mg/ml) it is not unreasonable that expression of

transferrin receptors would be under complex metabolic control, Neckers et al., (1983).

2. Molecular Characterization of BCGF and BCDF and Their Role In B-Cell Growth and Differentiation.

Maizel et al., (1983) using anti-mu activation have shown evidence of the effects of BCGF on a subset of cells thus to provide conditions supporting the long-term growth of these B lymphocytes. The long-term BCGF dependent populations provide an effective system for the sensitive accurate assessment of BCGF activity, and provide evidence for the presence of growth factor receptor on these cells.

The BCGF capable of stimulating human B cells to enter S phase of the cell cycle, so far has been characterized to be trypsin-sensitive protein with a molecular weight of 12,000-13,000. The protein has a mildly acidic isoelectric point (PI 6.3-6.6), is relatively unstable at 56°C, is unaffected by low concentrations of reducing agents, and is stable between pH 3 and pH 8. The human BCGF has a functional specificity in that its action is strictly proliferative. The first report of BCGF activity on human B cells was upon exposure to factor(s) present in media conditioned by lectin-stimulated mononuclear cells, Maizel et al. (1982). The conditioned medium from cells stimulated by lectin for 72 h was fractionated by ammonium sulfate precipitation, ion exchange chromatography and gel

filtration chromatography. T cell and B cell stimulatory factors present in the initial conditioned medium were found to copurify during ammonium sulfate precipitation, DEAE sephadex chromatography, and Bio-Gel P-30 gel filtration. However, partial separation of these two activities was achieved after Bio-Gel P-100 gel filtration. Analytic polyacrylamide gel electrophoresis of radiolabeled Bio-Gel P-100 column fractions demonstrated a distinct protein band of 14,000 - 15,000 daltons in those column fractions predominantly supporting T cell growth and a distinct protein band of 12,000 - 13,000 daltons for those fractions predominantly supporting B cell growth. However, Mehta et al., (1985) showed in contrast to human T cell growth factor, that BCGF appears to be derived from a putative precursor protein of 60,000 MW, Sahasrabuddhe et al., (1984), demonstrated by 17S mRNA species that codes for biologically active BCGF in Xenopus oocytes.

Another source for BCGF was reported by Kishimoto et al., (1984) and Okada , et al., (1983) to be T-cell hybridomas, peripheral T cells activated with protein A which were hybridized with azaquanine-resistant T leukemic cell line (CEM-AG). Establishment of human T hybridomas secreting BCGF or BCDF was also reported by other investigators. Butler et al., (1983) (1984a) and (1984b) have characterized the chemical and physical properties of

a monoclonal BCGF produced by human T hybridoma. They reported the molecule to be remarkably similar to human and murine IL-2 in terms of temperature stability and patterns of resistance to chemical denaturation and reduction. In further investigation of the cellular origin of BCGF and BCDF, Milanese et al., (1985) have shown that BCGF is produced by both T_4^+ and T_8^+ T cells at the population and clonal levels, while BCDF activity is largely but not exclusively, restricted to T_4^+ subsets, and both T_3-T_1 and T_{11} pathways activate individual clonal T cell populations to promote B cell growth and differentiation.

There is evidence for heterogeneity of BCGF from different sources of BCGF. Kaieda et al., (1982) have demonstrated the presence of two distinct kinds of BCGF by establishing an IL-2 dependent helper T-cell clone (d4) by MLC reaction against a B lymphoblastoid cell line (CESS). On stimulation with CESS cells d4 cells secreted several immunoregulatory molecules, and the culture supernatant showed IL-2, BCGF, and BCDF activities. BCGF activity from this particular cell line was eluted in the fraction of 50 K daltons. This showed a marked contrast with BCGF from PHA-stimulated normal T cells or from T hybrid clones, which had a molecular weight of 15,000 - 20,000. Stimulation of peripheral T cells with PHA plus PMA

(phorbol myristate acetate) induced both 20- and 50- K daltons BCGF, Yoshizaki et al., (1983). The addition of 50 K dalton BCGF from d4 cells to 20 K dalton BCGF from normal T cells synergistically augmented the proliferation of anti-u stimulated B cells, Kishimoto et al., (1983). Additional evidence of two human BCGF species separated by Con.A sepharose column was recently provided by Dugas et al., (1985), whereby the two biologically active molecules displayed different apparent MW and sugar content. Another source for high molecular weight (HMW) BCGF described by Ambrus et al., (1985a) was from supernatants of the human B lymphoma line, Namalva , and a human T cell line, T-ALL, and they were free of IL-1, IL-2, and IFN activity. More recently they have used these supernatants for the purification of HMW-BCGF to apparent homogeneity and subsequently they have produced a monoclonal antibody to the purified protein (Ambrus et al., 1985b). They have provided definitive evidence the HMW-BCGF is a lymphokine distinct from LMW-BCGF, or IL-2, both antigenically and functionally. Hence by using different sources of BCGF, and establishing monoclonal B-cell lines reactive either to BCGF or BCDF it was possible to reconstitute in vitro, the activation process of B cells , that is:

1) Cross linkage of Ig receptors activates B cells and induces receptors for BCGF

2) BCGFs induce the clonal proliferation of activated B cells

3) BCDFs induce final differentation of B cells into Ig-producing cells

The role of BCDF in biochemical and molecular analysis of B-cell activation, has been established through BCDF-reactive B-cell lines. Some biochemical processes induced by BCDF were revealed, that is,

i) binding of BCDF with BCDF regulators induced phospholipid methylation followed by serine esterase activation and,

ii) activated serine esterase was involved in the limited proteolysis of cytoplasmic precursors into the active substance, which may be responsible for the signal transmission from membrane to nucleus.

In his attempts to prepare monoclonal antibodies reactive with BCDF receptors, Kishimoto (1983) has selected and employed human B-mouse myeloma heterohybridomas that were depleted of many human cell-surface antigens but still retained BCDF receptors. In order to clone genes coding for human B-cell-specific antigens, including BCDF receptors, he has attempted to induce an expression of human B-cell antigens on murine L-cells by DNA-mediated gene transfer. High molecular weight DNAs from a

BCDF-reactive B-cell line were cotransferred with TK gene into TK-L cells, and transformed cells expressing human B-cell antigens were screened by a replica method with B-cell-specific monoclonal antibodies.

Hence isolation and characterization of factors and their receptors on target B cells will reveal the activation mechanism of B cells at the molecular level and open new approachs for the manipulation of several immune disorders, such as immunodeficiencies, autoimmune diseases, allergies.

Kishimoto (1985) in his latest review on the factors affecting B-cell growth has summarized the results obtained with conventional as well as hybridoma-derived factors. These results clearly demonstrate the presence of B-cell specific growth factor(s) (BCGF) involved in the induction of proliferation of B cells, that is,

a) BCGFs act on B cells triggered by anti-Ig, LPS, or SAC, but not on resting B cells;
b) receptors for BCGFs are expressed on activated B cells, but not on resting B cells, B cells at the final maturation stage, or activated T cells;
c) BCGFs are responsible for the induction of proliferation of B cells, but not for the induction of final maturation of B cells into Ig-secreting cells.

Through the attempt to isolate and purify BCGF from different sources led to an appreciation of the fact that human IL-2 and BCGF were quite similar biochemically, Muraguchi et al., (1982 b). It is now clear that human BCGF and IL-2 are indeed separate molecules. This is supported by several lines of evidence,

1) IL-2 dependent T cell lines can absorb IL-2 activity without diminishing the activity of BCGF in supernatants containing both factors, Maizel et al., (1982), Muraguchi et al., (1982 b), Yoshizaki et al., (1983).
2) Partial separation of IL-2 and BCGF activity has been achieved by the use of ammonium sulfate precipitation, ion-exchange chromatography, and gel filtration chromatography (from media conditioned by phytohemagglutinin-stimulated T cells, Maizel et al., (1982). Mehta et al.,(1985).
3) T-T hybridomas have been established which constitutively produce human BCGF in the absence of IL-2, Butler et al., (1983), Okada et al., (1983).

Nevertheless, there are still controversies whether BCGFs are really involved in the proliferation of B cells or are involved in the activation of B cells responsive to other growth factors not yet identified.

This view was first challenged by time course studies by Howard and Paul (1983) who have demonstrated that a delay of even 4 h in the addition of BSF-1-rich supernatants to resting B cells cultured with anti-IgM caused a diminution in the magnitude of entry into S phase at 30-42 h after initiation of culture; delays of 12 h or greater in the addition of BSF-1 completely abrogated the uptake of (^{3}H) thymidine between 30 and 42 h of culture. Such studies indicated that BSF-1 acted on very early G_1 phase or perhaps on G_o B cells. Recent study by Robin et al., (1985) has shown that the treatment of mouse B cells with BSF-1 also induces a state of activation, as shown by the fact that these cells are capable of entering S phase more promptly on subsequent culture with anti-IgM and BSF-1 rich EL-4 supernatant than are B cells precultured in medium only.

Previous data by Thompson et al., (1985) and Noelle et al., (1984) on induction of class II MHC molecules in resting B cells and increases in cell volume in such cells indicate that BSF-1 should be regarded as an activation factor. Whether it also has a biochemically distinct function as a growth-inducing factor or whether that activity reflects its initial activation properties and/or the continued generation of precisely the same intracellular signals as the cell moves through the G_1 phase are issues that require resolution.

3. IL-2 and IL-2 Receptor Interaction on B Cells

It is well established that IL-2 plays an important role in the proliferative response of T cells. Activated B cells were also recently found to express IL-2 receptors. However, it is still controversial whether IL-2 is involved in B cell growth and/or differentiation. The answer to this question is dependent on the stimulation agent used. A definitive answer to the question of BCGF and IL-2 being distinct molecules or molecules showing common epitopes which could be the target for signal processing for proliferation and differentiation, can not be answered unless the receptor for BCGF is isolated and cloned, probed, and comparative qualitative, quantitative, and functional studies done between IL-2 receptor and BCGF receptor on B cells and T cells. Suzuki and Cooper (1985) have investigated the expression of IL-2R on using phorbol myristate acetate (PMA) stimulated human B and T cells. Even though both T and B cells expressed IL-2R of the same molecular weight (54,000 to 59,000) regardless of the method of T or B cell activation, the same authors showed several differences in the mode of induction and the functional characteristics of IL-2R on T and B cells.

1) The B cell response with IL-2 receptor expression was delayed by approximately one day and was slightly less than for T cells.

2) IL-2 enhancement of T cell proliferative response was observed over a wide range of PMA concentrations, whereas enhancement of the B cell response was limited to a narrow range of PMA concentrations.

3) Higher concentrations of IL-2 were required for enhancement of the proliferative responses of PMA-activated B cells than for T cells.

4) Lower concentrations of the anti-Tac antibody were required for inhibition of the IL-2 enhanced proliferative responses of B cells.

5) Although the addition of IL-2 enhanced both the PMA-induced increase in T cell size and transferrin receptor expression, no demonstrable enhancement was observed for the B cell response.

Similar results were shown by Mitler et al., (1985). Using indirect fluorescence they have indicated the expression of Tac on SAC and T cell replacing factor-supernatant (TRF-SN) activated B cells obtained from peripheral blood, tonsil or spleen. Biochemical characterization of the Tac antigen derived from activated T and B cells in this system, showed them to be identical in MW and to be composed of identical methionine containing chymotryptic peptides. In addition, anti-Tac can inhibit both proliferation and the subsequent generation of plaque forming cells (PFCs).

a) Evidence for IL-2 Receptor Expression on B Cells

The question to be asked is, is IL-2 receptor expression a universal B cell response, or is it dependent on the mode of B cell activation?

The first report of the expression of the receptor for IL-2 on B cell as defined by the Tac antigen was done by Korsmeyer,et al., (1983). These authors described the presence of the Tac antigen on hairy cell leukemic cells which have a B cell phenotype as evidenced by re-arrangement and expression of immunoglobulin genes. Another evidence that IL-2 might play a role in B-cell maturation was suggested by Depper et al., (1983) by showing that anti-Tac inhibited the maturation of B cells into immunoglobulin-secreting cells as measured in a reverse hemolytic plaque assay when peripheral blood mononuclear cells were stimulated with pokeweed mitogen. Also, Waldmann et al., (1984) have demonstrated the presence of Tac antigen in leukemic B-cell populations, and cloned lines of normal, Epstein-Barr virus (EBV) transformed B cells. These cells were shown to display the Tac antigen and high affinity IL-2 receptors identified in binding studies with purified radiolabeled IL-2.

There is also evidence of the expression of the IL-2 receptor by the marginal zone B lymphocytes of the spleen which usually contains lymphocytes with an intermediate morphologic form between small lymphocytes and plasmablasts, Hsu (1985). Thus, IL-2 may play a role in the differentiation of activated B cells into Ig-synthesizing and -secreting cells. The Tac-positive B lymphocytes may represent a discrete subpopulation of B lymphocyte with unique immunologic function distinct from that of Tac-negative activated B cells. Accordingly, the conclusion from the studies by Hsu (1985) was that the Tac-positive marginal zone B cell system and Tac-negative germinal zone cell system may act independently with different immunologic mechanisms. The repopulation of B cells in the spleen after sublethal or focal radiation is quite different between the marginal zone (MG) and germinal center (GC) systems. Both MG and GC cells are derived from circulating B lymphocytes. The repopulation of the GC/MZ system can be facilitated by a second booster of antigens, whereas the repopulation of MG system cannot. Other studies also indicated that the MG B cells are likely the precursors of IgM-producing cells, whereas the GC cells are responsible for the production of IgG blasts; indicating that the MG B system represents a stage or stages of B-cell differentiation that is responsible for the IgM cell

production in the primary immune response, and the GC would be the structure of the secondary immune response.

Lowenthal et al., (1985) have investigated a detailed quantitative comparison of IL-2 receptor expression on activated B and T cells. Quantitative ^{3}H-IL-2 binding revealed that B blasts, like activated T cells express two classes of IL-2 binding sites; one has a high affinity for IL-2 and the other a 50-100-fold lower affinity. The ratio of the number of low-to-high affinity sites per cell for B blasts is within the range found for activated T cells; (1:10). Lowenthal et al., (1985) have shown more evidence that IL-2 could promote the growth of appropriately stimulated B and T cells by inhibition, at similar antibody concentrations, of IL-2 dependent proliferation of both cell types, and by immunoprecipitation from activated T and B cells of similar Mw bands.

The polyclonal mitogen SAC induced B cells enriched from peripheral blood to express functional IL-2R, as demonstrated by Tsudo et al., (1984). These investigators also showed that immunoaffinity purified IL-2 induced the proliferation of SAC-activated B cells, and the proliferation was completely inhibited by anti-Tac antibody, which blocked the membrane binding and action of IL-2. Whether IL-2 plays a similar functional role in B cell is still a debated question. Nevertheless, there is

evidence that IL-2 directly enhances B-cell proliferation and/or differentiation. Muraguchi et al., (1985) assessed the biological properties of the IL-2R on B cells by incubating B cells with recombinant IL-2, and have found that moderate concentrations of IL-2 induce significant enhancement of proliferation and differentiation in SAC-activated normal B cells. On the other hand, in spite of these, there are several lines of evidence that IL-2 may act directly on B cells. In studies of the synergistic effects of cofactors on B cell function, depletion of IL-2 from cofactor-containing supernatants by absorption on IL-2 dependent cells also removed factors that influence B-cell function, which suggests that IL-2 may be one of the cofactors affecting B cell responses. Results indicating that IL-2 can function as a BCGF were reinvestigated by Mingari et al., (1985). These workers used both affinity-purified and recombinant IL-2 and analysed their effect on the proliferation of purified human B cells activated with SAC. In addition, Mingari et al., (1984) have demonstrated that anti-Tac MAb reacts with SAC-activated normal B cells, and in addition, immunoprecipitation experiments revealed that the anti-Tac MAb defines similar molecules on activated T and B cells.

2. Functional Role of IL-2 in B Cell Growth and Differentiation.

Some functional studies on lymphokines suggested that carbohydrate structures on the receptors for lymphokines are responsible for the expression of their functions. In this context, Takahama et al., (1985) have shown the existence of GalNac and sialic acid residues on B151-TRF molecule which is T cell-replacing factor molecule derived from a monoclonal T cell hybridoma and the important role of the GalNac residue in the binding of TRF molecule to the corresponding B cell receptor.

Ando et al., (1985) used phorbol ester (TPA)-stimulated B cells to show that phorbol esters are able to substitute for all the three signals activation, proliferation and differentiation, but an increment in proliferation is obtained when IL-2 is added to phorbol ester stimulated B cells. The same authors have indicated that anti-Tac MAb could only reduce IL-2 stimulated B cell proliferation to the level produced by TPA induction alone implying that IL-2 is a proliferative signal acting by a different pathway to TPA in B cells.

It is still a subject of debate whether IL-2 plays an important role in the differentiation of B cells. To resolve this question, investigators have more recently, used recombinant IL-2. Ralph et al., (1984) used high concentrations of IL-2 directly stimulated the IgM and IgG production by cell lines. The concentrations used by these

authors were beyond any physiologic significance in B cell activation; also, cell lines do not represent the whole spectrum of normal B cell populations. However, using different anti-Tac antibodies and assaying for the Ig secretion on the same cell lines they have shown that IL-2 stimulates B cells via a low-affinity interaction with a receptor different from the Tac receptor identified on T cells, and that the active site on the IL-2 molecule for B cells differs from that for T cell targets. However, Kishi et al., (1985) used a cloned B-cell line and showed that IL-2 induced Ig secretion in B cells. Because IL-2 induced IgG secretion in SGB3 cells was inhibited by anti-Tac antibodies, activities of IL-2 to induce proliferation and to induce differentiation appear to be exerted through the same receptor molecule.

While the previous papers have shown a direct effect of IL-2 on differentiation of B-cell lines, or cloned B-cell line, Teronishi et al., (1984) have shown the involvement of IL-2 in the differentiation of SAC stimulated B cells to IgG-producing cells unless either small number of T cells or the late acting BCDF coexisted.

In further delineating the role that IL-2 could play in B-cell activation and/or differentiation, Mond et al., (1985) have shown that affinity purified IL-2 induces proliferation of large but not small B cells of mouse

origin. They also have shown that the proliferation inducing capacities of BCGF and IL-2 were not additive, which suggests that these two factors probably do not exert their effects on different B-cell subpopulations. Although BCGF and IL-2 appear to stimulate proliferation of the same subset of large B cells, they do so via two distinct modes of activation. Whereas BCGF in nonlimiting amounts was unable to sustain a continued increase in B cell proliferation above that seen on day 3 of culture, B cell proliferation in response to IL-2 was markedly higher by day 4 of the culture period. Similar observations were reported by (Boyd et al., 1985 and Almerigogna et al., 1985) who investigated the kinetics of the human B cell response to growth factor and showed that BCGF acts within 24 h, whereas IL-2 was virtually devoid of activity for 48 h. Nevertheless, after 72 to 96 hr the effect of IL-2 was equal to or greater than that obtained with BCGF. These reports support the notion that activated B cells express distinct receptors for BCGF and IL-2. This observation that both BCGF and IL-2 induced activated B cells to proliferate but that only IL-2 inhibited by anti-IL-2 receptor antibody suggests that these are distinct receptors.

These studies suggest that B cell proliferation is determined by sequential interaction of BCGF and IL-2 with their respective receptors. Pike et al., (1984) have used recombinant IL-2 to investigate the number, molecular characteristics, and modes of action of B-cell growth and differentiation factors, they have assayed recombinant IL-2. IL-2 in a unique B lymphocyte cloning system in which a single, hapten-specific murine B cell can be placed into culture (in the absence of any accessory or feeder cell) and stimulated with a combination of specific antigen and B cell active lymphokines to divide and secrete antibody. In such a system they have shown that r-IL-2 does possess B-cell growth and differentiation-promoting activity.

Zubler et al., (1984) considering murine B cells activated by LPS and anti-Ig, a system for optimal induction for growth factor responsiveness, have investigated that either recombinant or immunoaffinity purified human IL-2 stimulated the proliferation of murine B cells in their system of study to the same extent as various T cell and spleen cell SN,, and that very similar IL-2 concentrations were required for B vs T cell proliferation, and that LPS plus anti-Ig-activated B cells expressed IL-2 receptors, a mean of 3500 IL-2 receptors per cell. With an apparent dissociation constant of 150 pM

this study was done by analysis of the binding of radiolabeled immunoaffinity purified IL-2.

In retrospect there is evidence of IL-2 receptor expression on activated B cell but whether it plays any immunomodulating role in B cell growth and development stays open for discussion.

III. CONCLUSIONS.

One of the complex and controversial areas of research in Immunology is the mechanisms of B lymphocyte development and activation. I should emphasize that the information gathered in the literature review about this area is derived from many divergent experimental systems where the assumptions vary and the conclusions derived from one model system are often difficult to reconcile with those derived from other model systems. Subsequently, in the first part of the project it was necessary to delineate the various parameters of the system chosen, and mode of stimulation of B cells for the most efficient signal for proliferation. I used mainly the lymphokines IL-2 and BCGF to investigate the role they might play in controlling the signalling mechanism by which anti-Ig drives B cells to undergo Go to G_1 transition, in relationship with activation antigens which might play an important role in the processing of the signals. Many different systems have been investigated to

study the role lymphokines might play in independently regulating cell growth and differentiation. Among those that have been described that enhance the proliferation of B lymphocytes include IL-1, IL-2, interferon (IFN), and several BCGFs. However, the precise molecular distinctions among these factors and the exact role that each plays in B cell function remains unclear.

Many questions remain unanswered in this area of endeavor: what is the role of BCGF in B cell proliferation? Is it independent of the expression of certain activation antigens or does it correlate with the expression of other activation antigens? In relation to this hypothesis is BCGF acting by ways of a specific BCGF receptor? or is it acting by way of the IL-2 receptor? or on a whole complex of antigens which are expressed upon activation of B cells? Is there an onset of a chain of a whole set of lymphokine gene activation, following the triggering signal by BCGF? To be able to answer all those questions is beyond the scope of this master's project. Therefore, in this thesis, I wish to ask two very simple questions concerning the mechanism of action of BCGF in stimulating B cell proliferation.

1. Is BCGF acting cooperatively with other cell surface molecules?

2. Is BCGF the only signal required for B cell proliferation or ultimately does BCGF activate the genes for receptors of a variety of other growth factor mainly the IL-2 receptor, concomitantly interact in the processing of the proliferation signal?

MATERIALS AND METHODS

1.Reagents

B cell growth Factor (BCGF) was obtained from Cellular Products Inc. (Buffalo, New York 14202). It was purified from the supernatant of human T lymphocytes stimulated with PHA-P in the presence of heat-inactivated human AB serum. The purification procedures they have used involved a series of column chromatographic steps incorporating ion exchange, affinity binding and gel filtration. BCGF was used at a 1:10 final dilution throughout the experiment.

Recombinant human IL-2 (rIL-2) was obtained from Chyron Co., Emmeryville. It was HPLC purified.

Staphylococcus Aureus Cowan 1 strain (SAC) in a 10% v/v suspension (Pansorbin) was purchased from Calbiochem-Behring (La Jolla, California). Pansorbin was treated as described by Schurman et al., 1980) and Wrigley and Choi (1983). It was briefly reformalinized in 1.4% formaldehyde for 60 min and boiled for 5 min. It was thoroughly washed and resuspended at a final concentration of 10% v/v in RPMI 1640 + 10% FCS and stored frozen at -20°C. The treatment was done to eliminate the shedding of Protein A and reduce its binding capacity for IgG. The optimal concentration of SAC for B cell proliferation as

measured by ^{3}H thymidine incorporation) was a 1:10,00 dilution.

2.Cell Sources and Cell Separation

Mononuclear cells (MNC) from peripheral blood or spleen were obtained by Ficoll-Hypaque density gradient centrifugation. About 50 ml of buffy coat obtained from Red Cross is diluted 1:2 with RPMI 1640 + 5% FCS and layered onto a lymphocyte separation medium (Isolymph, a Ficoll-sodium metrizoate solution of density 1.077, obtained from Pharmacia), at 2 volumes of diluted blood on 1 volume of Isolymph solution. Spleen cells which were obtained frozen from the transplant immunology laboratories from the University of Alberta Hospital. The cells were diluted in calcium-free buffered medium without FCS to minimize cell clumping. The tubes were centrifuged for 20 min at 20°C with an interphase force of 1000g. The cells of the interphase were collected carefully and pooled, and their suspension was diluted at least 5x with RPMI 1640 + 5% FCS and centrifuged at 400 g for 15 min. at 20°C, two further washings were done at the same force (400 g) for 10 min. Blood monocytes were depleted by culturing MNC in plastic culture plates for 1 h at 37°C.

3. BCGF Assay

Human B cells were purified as described in previous paragraph from peripheral blood or spleen. Purified BCGF obtained from Cytokine Technology International which was tested for IL-2 activity on mouse thymocytes which were IL-2 dependent for proliferation and also on IL-2 dependent mouse T cell line, both tests showed negative results. Then BCGF 1/10 final dilution was added to B cells (0.5 x 10^6/ml) in 36-well, flat-bottomed microtiter plates Linbro from Flow Laboratories Cat. No. 76-032-05) which were coated overnight with 10ug/ml of $F(ab')_2$ goat anti-human IgM heavy chain-specific anti-u (Cappel Worthington Biochemicals, PA 19355). Cells were cultured, and proliferation was measured by adding 1 uCi of [^{3}H] thymidine to each well and after 4 h of cell incubation, cells were harvested and [^{3}H] thymidine incorporation measured.

The average yield of PBLs is 98.9% (range 36-82%) or an average of 100 x 10^5/ml buffy coat. The viability ranged over 97-98%.

4. Proliferative Assay

Cell cultures were pulsed with 1 uCi [^{3}H] thymidine (specific activity 20 Ci/mM, New England Nuclear, Ontario, Canada) for 4 h before harvest kinetic study was done. Incorporation of ^{3}H-TdR was measured by a standard liquid scintillation counting technique after harvesting

cells onto glass fiber filters using an automatic titertech cell harvester (Flow). The dried filters were added to scintillation vials containing 2-3 ml of Toluene with 1.38g of Omnifluor/litre and counted in a RackBeta 1218 (LKB, Wallac, Finland) counter.

Thymidine uptake is expressed as mean counts per minute (cpm)+/- standard error (SE) from triplicate wells.

In inhibition experiments with anti-Tac antibody, suppression of the IL-2 synergistic effect was calculated as follows:

$$\% \text{ suppression} = \frac{\text{1-cpm (cultured with BCGF, rIL-2, and anti-Tac antibody)}}{\text{cpm (cultured with BCGF and rIL-2) - cpm (cultured with BCGF)}}$$

(a) **Isolation of T and B Lymphocytes**

The B lymphocytes enrichment was done by depleting PBLs of E-rosette-forming T lymphocytes, since T lymphocytes carry a receptor for sheep red blood cells (SRBCs) which allows the formation of E rosettes. Pretreatment of the SRBCs with 2-aminoethylisothiouronium bromide hydrobromide (AET) using a modification of the method of Madsen et al., (1980) was followed since this method allows to obtain stable E rosettes in a shorter time. Briefly, 2 ml of packed SRBCs are resuspended in 10 ml of 140 mM AET solution, pH 9, and incubated at 37°C for 30 min with occasional shaking. After incubation, AET-treated SRBCs

(AET-SRBCs) are washed until any residual hemolysis is observed. Finally, packed treated red cells are resuspended in RPMI 1640 medium with 10% FBS to obtain a 4% suspension.

T lymphocytes (E+PBLs) were isolated from the non-adherent MNC fraction by rosette formation with AET-SRBCs using a modification of the method by Pellegrino et al., (1975). This procedure was repeated twice and further treatment with pooled monoclonal antibodies OKT3 and 4F2 and lysis with complement was done, to get rid of most residual T cells.

5. Staining Procedures

(a) FITC staining

The cells to be tested for surface marker expression using indirect method of staining are first checked for viability. Usually cells must be 90% viable, otherwise they are passed through ficoll gradient to get rid of the dead cells and debris. The cells are washed once with cold medium RPMI + 2% FCS + 0.1% NaN_3 and incubated for 30 min with 50% human AB serum (which is tested for non-cytotoxicity) on ice to prevent Fc non-specific binding. The cells are washed twice with same washing medium by centrifuging for 4 min each at 400 g. After the third wash they are suspended in RPMI medium containing 2% FCS, 0.1% NaN_3 and 10% human AB serum. Meanwhile,

microtiter plate, 96 V bottom wells are soaked for 30 min. at 4°C with PBS + 1% BSA to block nonspecific binding. To proceed the staining 50 ul of cell suspension containing 1 x 10^6 - 0.5 x 10^6 cells were transferred into the BSA blocked wells, and 50 ul of primary antibody in ascitic form diluted 1:250 in PBS, 0.02% NaN_3 and 10% human AB serum, are added to the cells and the following reaction mixture is incubated for 30 min at 4°C for each set of surface markers tested. Appropriate controls are used for positive controls (usually HLA Class I marker is used) and negative controls (the same medium as the cells are suspended in).

After 30 min of incubation, the reaction mixture is washed 3x with same cold washing medium as mentioned above, by centrifuging 4 min each at 400 g. After last wash the pellets are suspended in 100 ul of 1:50 dilution in PBS + 0.02% NaN_3+10% AB serum of secondary antibody. FITC conjugated $F(ab')_2$ fragment of goat anti-mouse immunoglobulins (IgA + IgG + IgM) heavy and light chain specific (from Cappel Laboratories, Cochranville, PA) and incubated for 45 min at 4°C. The secondary reaction mixture is stopped by washing 3 times as mentioned above and the cells are transferred into nunc cryotube in 500 ul of 1% w/w formaldehyde solution (formaldehyde solution 37% w/w, Fisher Scientific Co.) in PBS. The samples were

analysed by a Coulter EPICS V flow cytometer using a 5 watt argon laser, and at excitation beam of 488 nm.

(b) Propidium Iodide/FITC Staining

This staining procedure was modified from Crissman and Steinkamp (1982) according to Traganos (personal communications). The same procedure as FITC staining is followed with the exception that after the primary antibody incubation the cells are fixed with 80% Ethanol in RPMI on ice for 1h before proceeding onto the secondary FITC conjugated antibody labeling.

To proceed onto the DNA analysis the pellet of cells, after washing, is suspended into 1 ml of propidium iodide (PI), RNAse mixture for 10 min, in the dark at room temperature. Then the cells are examined simultaneously for DNA profile and all surface antigen expression using FACS analysis.

PI, RNAse staining solution is each time freshly prepared. Propidium iodide (Sigma No. p-5264) 10 ug/ml in HBSS and RNAase (Worthington No.5679 3002u/mg) 2000 units/ml. These two solutions are mixed in a 1:1 proportion and 1 ml of the mixture added to 10^6 cell pellet.

6. Flow Cytometry Analysis

The cells which were stained indirectly for cell surface markers were examined using fluorescent activated cell sorting (FACS) analysis, using EPICS V cell sorter (Coulter Electronics, Hialeah, Florida). Before each analysis, the instrument settings were standardized by analysis of fluorescent beads. Samples were then analyzed at a constant gain setting which was set according to positive and negative cell populations, and the results were displayed on a histogram with a 256 channel abscissa representing the intensity of green fluorescence, through a multiple data aquisition display system (MDADS). The fluorescence signal is logarithmically amplified over 3 decades on a 256 channel abscissa. An analysis of the transfer function through the logarithmic converter indicated that an increase of 25.6 channels represents a doubling of the fluorescent intensity. The relative fluorescent index (RFI) is used to convert the logarithmically amplified scale to a linear scale, in order to compare the test sample to its control. Expressing results, as a percentage positive only, obscures the quantitative differences among the cells with respect to antigen density. The Coulter EPICS V flow cytometer is capable of achieving a quantitative estimate of the amount of antigen present via the RFI.

$$RFI = 2^{\frac{(\text{Channel no. of mean LIGFL (test sample)}) - (\text{Channel no. of mean LIGFL control sample})}{25.6}}$$

According to Weeks et al. (1984).

7. **Dot Hybridization Analysis of Cellular Cytoplasmic Preparations. White and Bancroft (1982)**

mRNA content of B cells given different signals of activation and/or proliferation was quantitated by using the dot blot technique which measures the extent of hybridization of cloned cDNA fragments to cytoplasmic mRNA $3x10^6$ cells were pelleted by centrifugation (600xg, 10 min), resuspended in 1.5 ml sterile phosphate-buffered salts, and repelleted by centrifugation in a sterile Eppendorf tube (15000 g for 15 s at 4°C). After resuspension in 45 ul of ice-cold 10 mM Tris (pH 8.0), 1 mM EDTA, cells were lysed by addition of two 5-ul aliquots of 5% Nonidet NP.40 with 5 min mixing on ice in between. Following pelleting of nuclei (15000xg, 2 min), 50 ul of the supernatant were transfered to a sterile 1.5 ml Eppendorf tube containing 30 u of 20x SSC [(standard saline citrate) 0.15 M NaCl/0.015 M trisodium citrate] plus 20 ul of 37% w/w formaldehyde . The mixture was then incubated at 60°C for 15 min, and stored at -70°C.

For analysis, 100 ul of each sample were serially diluted up to 1:64 dilution with 15x SSC in a 36 well microtitre plate to yield a final volume of 100 ul of each dilution; these were applied with suction to a 4 mm

diameter spot on a nitrocellulose sheet (BA85, 0.45 um) supported on a No. 470 paper employing a 36-hole Minifold apparatus, all from Schleicher and Schnell. The nitrocellulose sheet was then baked (80°C, 90 min) in vacuo to fix cytoplasmic macromolecules. Prehybridization of the nitrocellulose, preparation by nick translation of the ^{32}P-labeled probe (specific activity, 20X 10^5cpm/mg) hybridization, autoradiography, were performed as described in the following paragraph.

8. **Northern Blotting**

Four different groups of B cells which were treated under different stimulation conditions were lysed in 4M Guanidine Isothiocyanate and the total mRNA was separated from the cellular debris by centrifugation on Cesium Chloride gradient, following the procedure by Chirgwin et al., 1978). Gels for northern analysis of mRNA consisted of 0.8% agarose (Biorad) in buffer containing 20 mM MOPS (3-[N-mopholino]propanesulfonic acid). pH 7.0 - 5 mM NaOAC - 1 mM EDTA, 1% formaldehyde and 1.5 ug/ml ethidium bromide. RNA samples (6-12 ug) were incubated at 55°C for 15 min in 20 ul of the same buffer containing 6.5% formaldehyde and 50% deionized formamide, before being cooled to 4°C and loaded onto the gel. Gels were run for about 10 h at 30 volts, soaked in 20x SSC for 1 h and then baked following the same procedure mentioned in previous paragraph.

Filters were prehybridized for 6-18 h at 42°C for full length cDNA probe in 50% formamide, 50 mM sodium phosphate, pH 6.8, 5x Denhardts solution, 5x SSC, 2.5 mM EDTA, 2 mM sodium pyrophosphate (100 uM ATP, 100 ug/ml heat denatured salmon sperm DNA and 100 ug/ml yeast tRNA, Maniatis et al., (1982). Hybridization to ^{32}P-labeled cDNA probe for human IL-2 receptor which was generously provided by Dr. Chris Bleakley, was carried out in the same solution for 18 h at 42°C. Filters were washed for 1 h at 42°C, in 2xSSC-0.1%SDS followed by 2 weeks for 15 min at 55°C in 0.2xSSC - 0.1%SDS, processed for autoradiography.

The cDNA probes prepared according to Shaw et al., personal communications, were nick translated using DNA polymerase to a specific activity of 2.7 - 4.8 x 10^8 dpm/ug and used at 1.5 - 3 x 10^6 dpm/ml.

RESULTS

I. DEFINITION OF THE BEST B CELL ACTIVATION AND PROLIFERATION SYSTEM

Table I summarizes the criteria I used in defining the best system of *in vitro* B cell activation and proliferation where I could further investigate the relationship of BCGF and IL-2 in B cell function.

I have used different mitogens and T independent antigens for B cell activation. Among the mitogens used in the analysis of B cell function were pokeweed mitogen, staphylococcus aureus Cowan I strain, EBV, LPS, and anti-mu (data not shown), and based on these criteria the best activation system chosen and used throughout the study was human B cells activated with goat anti-human IgM $F(ab')_2$ (anti-mu heavy chain specific). The best signal for proliferation was found to be with BCGF purified from PHA stimulated T cells.

The optimal conditions of BCGF after anti-mu activation was found to be 1:10 final dilution. Figure 1 illustrates the checkerboard titration between different concentrations of BCGF and anti-mu

Table I. Summary of Criteria for Choosing the System of B Cell Activation for Further Investigation of the Correlation of the Mechanism of Action Between BCGF and IL-2 in B Cell Function.

1. Intensive purification of the B cell population.

2. Assessment of a good signal of activation and of proliferation by kinetic study of the profile of transition of B cells from G_o to S based on qualitative and quantitative results of:

   a) ^3H thymidine incoporation

   b) propidium iodide stainfng where the DNA content correlated with cycling cells

   c) kinetics of density of selected activation antigens such as anti-DR, 50H19, transferrin receptor and 4F2 based on RFI parameter

3. Pure source of factors :recombinant IL-2 and purified BCGF. The purification involved a series of column chromatographic steps incorporating ion exchange, affinity binding and gel filtration. So the best mode of stimulation of B cells which correlated with highest proliferative response in parallel with highest density on the surface of the above-mentioned antigens was B cells which were activated with anti-mu and after 24 h they were given BCGF stimulus for proliferation signal.

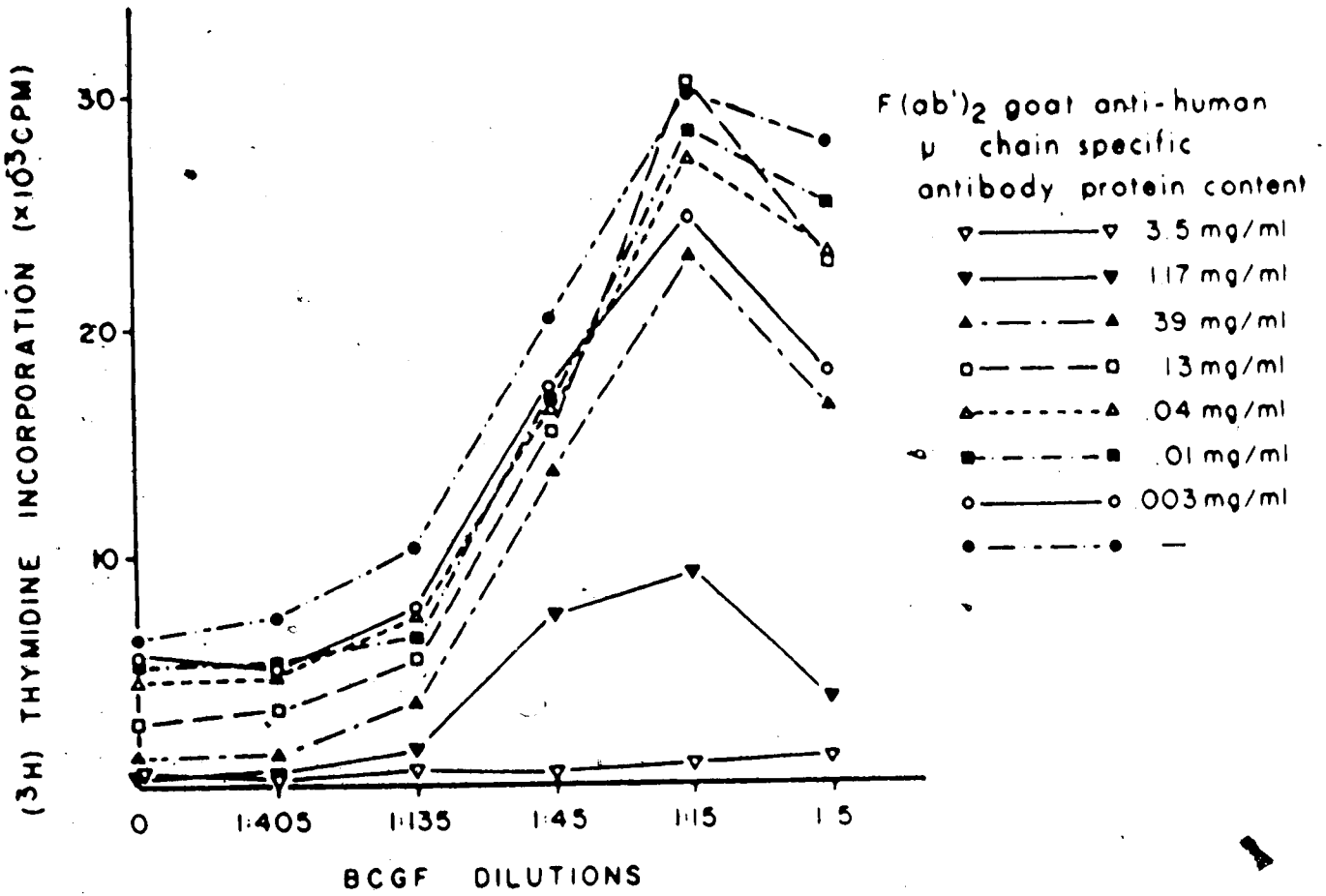

Figure 1. Checkerboard titration of BCGF and anti-mu concentrations

B cells at 10^5 cells/well were added to microwells coated with various concentrations of anti IgM. Different dilutions of BCGF were added at 24 hours and ^3H thymidine incorporation measured 3 days later. The cpm are means of triplicate cultures

Table II. Kinetics of Proliferation Profile of the Following Systems: Control, BCGF + Anti-mu, SAC, IL-2, or PHA

	D1	D3	D4	D5	D7	D8
Control	408	798	1227	58	82	113
BCGF + IL-2	1164	6612	3448	4546	3846	3644
BCGF	1722	7224	4916	3748	4549	3334
BCGF + SAC	1391	6060	7266	6440	5186	7348
STA	518	1775	1774	570	115	121
BCGF + SAC + IL-2	845	3744	4116	6834	3378	4137
PHA	102	77	44	56	68	88
SAC + IL-2	324	343	758	492	74	108

Each number represents the mean cpm value of triplicates

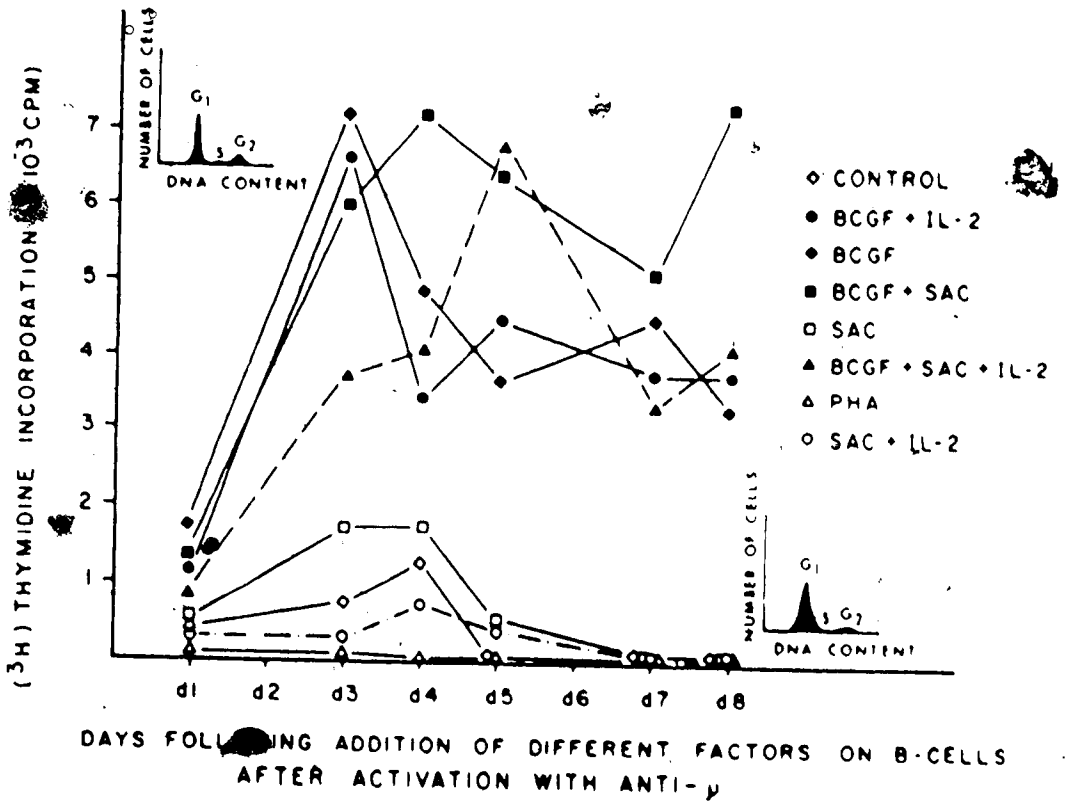

Figure 2. Time course kinetic study at proliferation profile of human B cells exposed to different stimuli.

Human B cells (2×10^5 well) were cultured for $F(ab')_2$ with anti-IgM mu chain specific 150 ug/ml coated microwells and after 24 hours the following were added alone, or in combination: BCGF (1:10), recombinant IL-2 (50 units/ml), SAC 1:10000. [^{3}H] thymidine incorporation in parallel cultures was measured every 24 h for 8 days after adding the factors. Four days after adding the factors propidium iodide staining was done on each set/group of cultures and DNA content was measured by flow cytometry.

Table III. Kinetic Study of the Effects of IL-2 and Anti-Tac on B Cell Proliferation Induced by BCGF After Anti-mu Activation

Stimulus and Factors Added	[^{3}H] Thymidine Incorporation (cpm) ± SD					
	Day 1	Day 3	Day 4	Day 5	Day 6	Day 8
Anti-mu	678 ± 44	350 ± 77	311 ± 65	324 ± 130	2128 ± 607	555 ± 121
Anti-mu + BCGF	1919 ± 192	5886 ± 85	9016 ± 1220	10356 ± 843	13661 ± 2568	7970 ± 913
Anti-mu + BCGF + anti-Tac D0	672 ± 138	2364 ± 405	3793 ± 659	6183 ± 240	8940 ± 3779	8532 ± 881
Anti-mu + BCGF + anti-Tac D2	1849 ± 739	2351 ± 131	2290 ± 230	4095 ± 122	6274 ± 788	6636 ± 1161
Anti-mu + BCGF + anti-Tacd4	1606 ± 108	7555 ± 614	6043 ± 500	2815 ± 291	4160 ± 1185	7604 ± 427
Anti-mu + BCGF + IL-2	4022 ± 453	9326 ± 1099	13828 ± 1548	17814 ± 1343	24136 ± 1982	28654 ± 1840
Anti-mu + BCGF + IL-2 + Anti-Tac D4	2350 ± 222	11456 ± 1616	8467 ± 389	4612 ± 344	9016 ± 3650	5621 ± 804
Anti-mu + anti-Tac	559 ± 284	190 ± 45	253 ± 56	639 ± 162	714 ± 157	552 ± 272
Anti-mu + IL-2	1530 ± 183	1396 ± 340	2185 ± 793	2369 ± 714	2448 ± 511	1612 ± 212
Anti-Tac	238 ± 19	198 ± 23	236 ± 38	497 ± 310	630 ± 218	310 ± 143
BCGF	2525 ± 809	5460 ± 1097	9597 ± 652	11946 ± 1635	12442 ± 494	12865 ± 2707
IL-2	806 ± 150	1680 ± 60	2486 ± 621	3014 ± 631	6538 ± 772	3324 ± 125
Control	-	302 ± 56	243 ± 46	408 ± 124	974 ± 360	335 ± 162

Looking at the profile of the kinetics of proliferation in Figure 2 and TableII, marked differences were noted for different stimuli. The peak of proliferation for B cells given the BCGF stimulus after anti-u activation was day 3, the same as when BCGF was given in combination with recombinant human IL-2.

SAC alone was not sufficient to give the proliferative signal for B cells to undergo transition into S phase (Figure 2).

II. INTERRELATIONSHIP OF BCGF AND IL-2 IN B CELL PROLIFERATION

1. Synergistic Effects of IL-2 on anti mu and BCGF Stimulated B Cells

The major question asked in the following set of experiments is whether BCGF and IL-2 cooperate together in the procession of the outgrowth of the proliferation signal in B cells?

This was examined using a checkerboard titration between BCGF and IL-2 on anti-mu activated B cells. Since the addition of IL-2 increased the ^{3}H thymidine incorporation (figure 4). An optimal effect was seen when 15 units/ml of HPLC purified IL-2 was added to B cells stimulated by BCGF in 1:10 dilution .

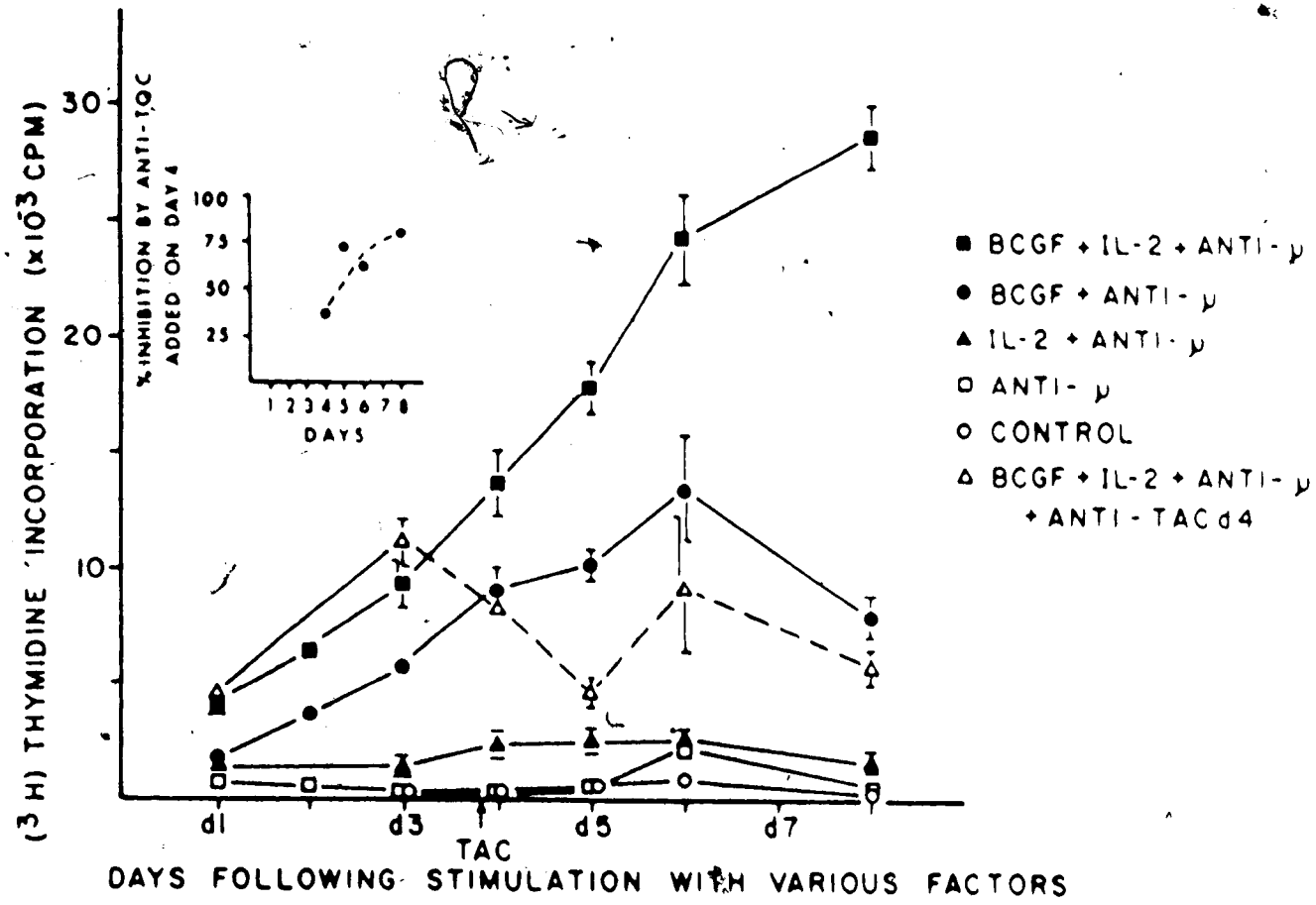

Figure 3. Time course kinetic study of synergistic effects of recombinant human IL-2 or/and anti-Tac on BCGF stimulated human B cell proliferation.

Human B cells (10^5 well) were cultured on anti-mu coated microwells (150 ug/ml) for 24 hours, then BCGF (1:10 dilution) + IL-2 at a final concentration of (25 units/ml), were added either alone or together. Anti Tac (Pool 39C6.5 + 33B3.1) was added on day 4 and ^3H thymidine incorporation was measured every 24 h for 8 days after adding the factors.

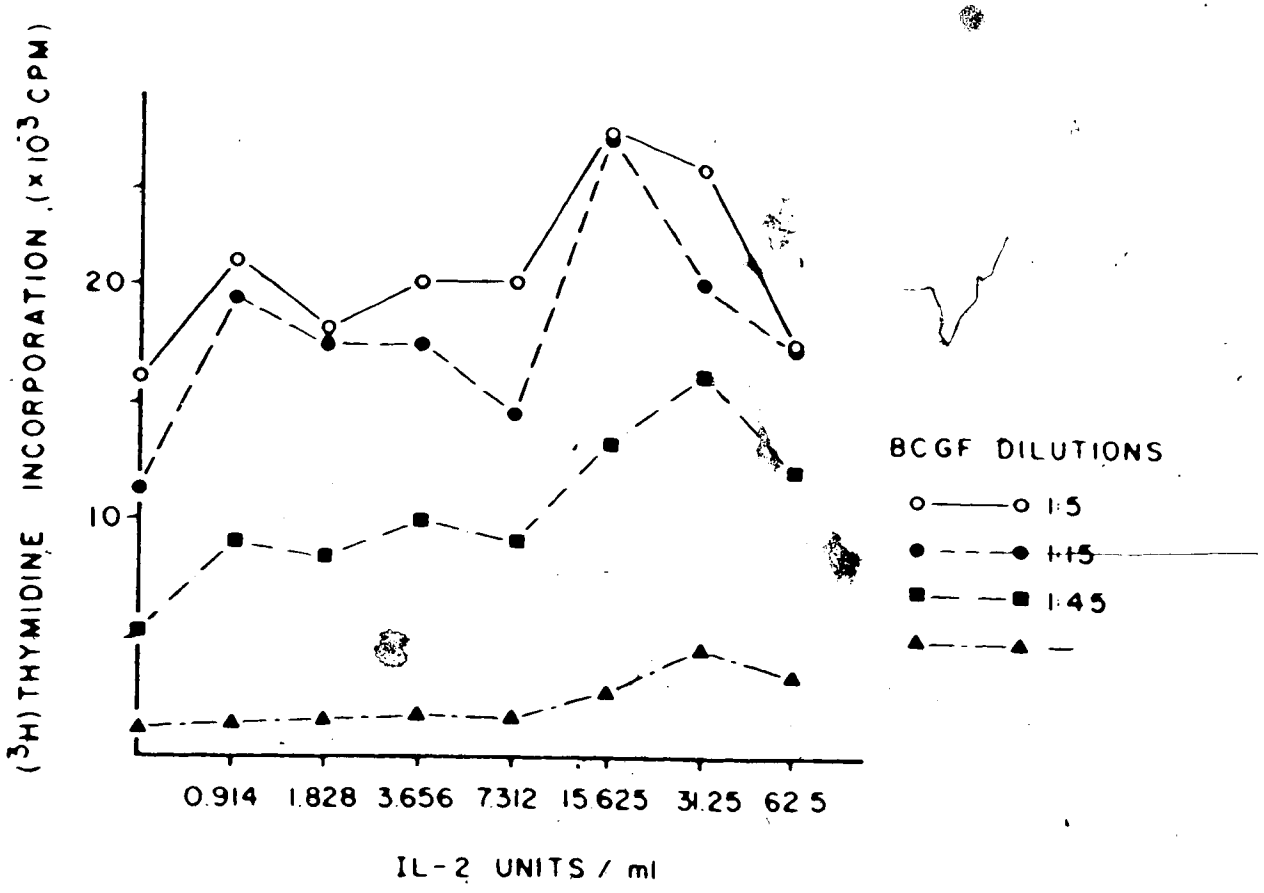

Figure 4. Checkerboard titrations of human recombinant IL-2 and BCGF proliferation effects of anti-mu activated human B cells from PBL of normal donors.

B cells at 10^5 cells/well were added to microwells coated with anti-IgM (25 ug ml) for 24 hours. Different concentrations of both recombinant human IL-2 and BCGF were added to the cultures and ^{3}H thymidine incorporation measured 3 days later. The cpm are means of triplicate cultures.

Table IV Anti-Tac Expression on Human B Cells Induced With Factors for Proliferation After Day 4

FACTORS	SURFACE ANTIGENS											
	3B7 3		39C6 5		7H3		5OH19		IgG		PBS	
	%LIGF	RFI	%LIGF	RFI	%LIGF	RFI	%LIGF	RFI	%LIGF	RFI	%LIGF	RFI
Control	15	1 0	15	1 05	33	-	39	10 83	95	7 62	10	1
BCGF+IL-2	15	1 17	44	0 55	60	14.6	55	11 13	80	9 98	8	1
BCGF+SAC	11	0 72	49	3 29	52	4 8	-	-	81	9 2	8	1
BCGF	16	1 0	18	1 05	69	9 98	73	17 16	92	9 2	11	1
SAC	17	0 9	15	0 87	45	0 95	52	10 26	-	-	9	1

Table V. Kinetics of the Variation of Tac Expression on Human B Cell Given Different Lymphokines

Resting	B Cells Stimulated with Anti-mu						
	Factors						
	BCGF				BCGF + IL-2		
MAb	% Positive Fluorescent Cells						
	D0	D2	D4	D6	D2	D4	D6
B4	42	32	27	21	36	27	14
B5	40	46	37	34	46	34	18
BK19	13	46	54	64	42	47	55
39C65	16	10	16	16	13	14	21
3387.3	14	9	15	20	13	15	21
10G33	18	29	22	24	31	22	24
BA1	44	44	28	45	47	28	24
7H3	45	57	54	62	61	55	65
41H19	46	56	32	44	55	40	48
4F2	38	65	54	73	64	53	74
5OH19	19	45	46	54	43	42	49
3383.1	8	10	18	65	13	19	67
39C1.5	22	20	19	27	17	27	36
OKT3	29	25	25	23	25	35	32
M18	99	98	95	96	99	90	99
IgG	28	57	64	5	60	57	8
PolyIg	71	93	86	47	92	80	46
PBS	6	6	10	7	11	16	14

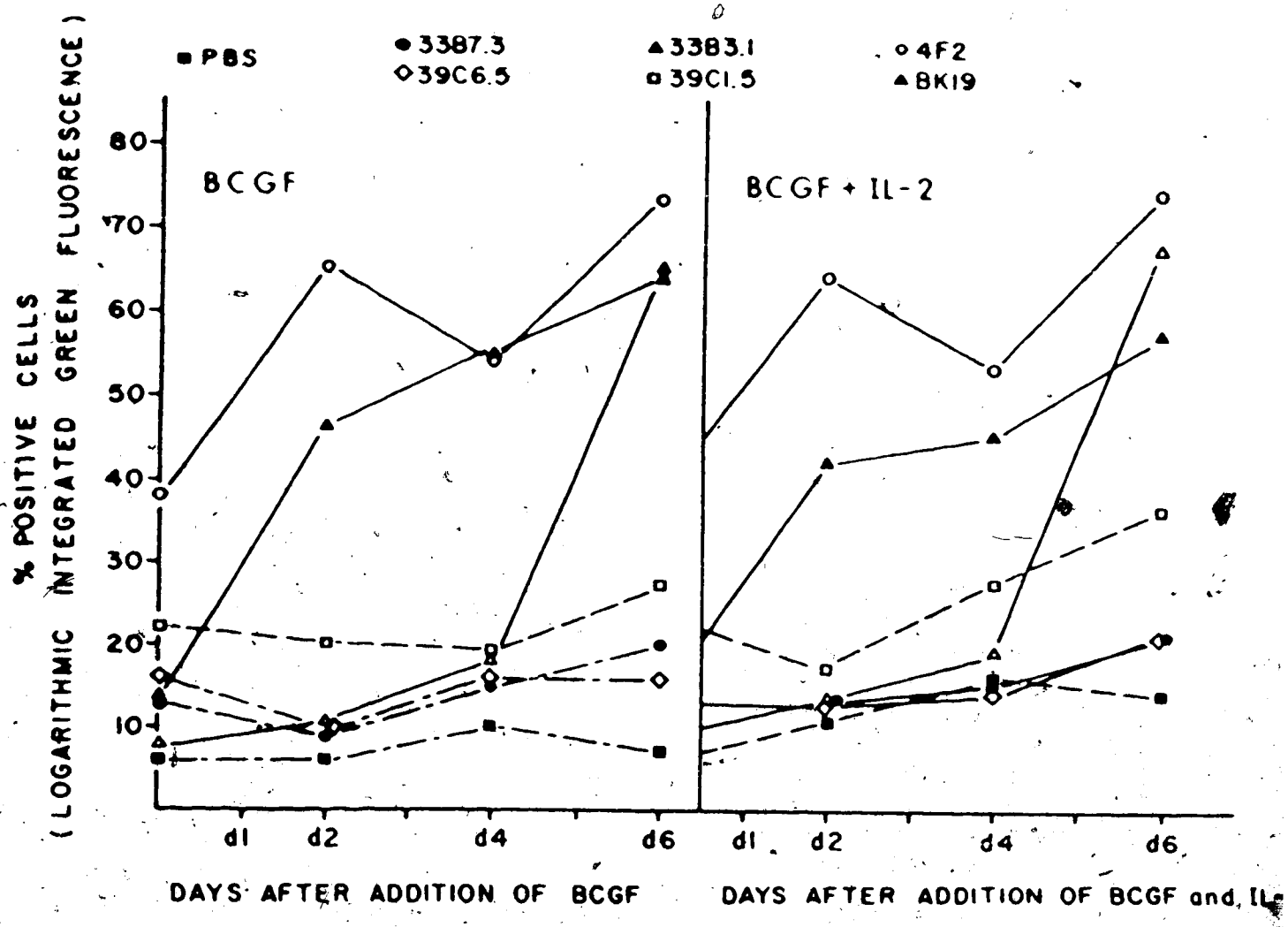

Figure 5. Comparative study at the kinetics of Tac expression on B cells stimulated with BCGF or BCGF + IL-2.

5 x 10^6 B cells were cultured (10^6/ml) in 5 cm diameter costar plates coated with anti-mu (25 ug/ml). After 24 hours optimal concentrations of BCGF or BCGF and IL-2 were added to the cultures. At various times after the addition of factors the expression of different Tac antigens was examined by indirect immunofluorescence and flow cytometry. Non-stimulated and dead cells were gated out on the basis of forward light scatter. 10^4 cells were analyzed and the

The time course study was done to investigate the effect of recombinant human IL-2 on anti-mu activated B cells stimulated with BCGF, as shown in Figure 3 and data summarized in Table III. IL-2 alone did not have any effect, but in combination with BCGF there was an increment of proliferation measured by ^{3}H thymidine incorporation.

2.Inhibition by IL-2 receptor MAb.

In further assessing the effect of IL-2 whether it was through the interaction with IL-2R, the following experiment was done. A pool of Tac monoclonal antibodies were added on day 3 and there was significant inhibition of ^{3}H thymidine incorporation. This additive effect of IL-2 was observed in 6 experiments but there was variation from one donor to the other. These results were in agreement with those of Almerigogna et al., (1985).

In the next experiments the question which was asked was whether the additive response of IL-2 and BCGF on B cell proliferation is dependent on IL-2 receptor expression. To attempt to answer this question three approaches were taken.

a)Is there Tac expression on the surface of B cells? On which subset of B cells and when is there Tac expression?

The second approach for the evidence of the role IL-2 plays in B cell function was to look at anti-Tac expression on B cells stimulated with different factors, by using FACS analysis. As in the litterature I could show evidence for the IL-2 receptor on B cells upon activation with anti-mu and subsequently given the signal of proliferation as shown in Table IV. The system where there was highest increase in Tac expression was B cells given simultaneously SAC and BCGF signals following anti-mu activation (data not shown). When different sources of B cells were used spleen cells did show higher expression of anti-Tac when given SAC and BCGF signal, even though this system does not correlate with the highest DNA synthesis profile, and also it shows low DR expression.

On the other hand Figure 5 and Table V illustrate comparative study of the kinetics of Tac expression between B cells stimulated with BCGF and BCGF and IL-2 together. Both systems of stimulation showed comparable Tac expression. Between the four anti-Tac monoclonal antibodies 33B3.1 showed the highest increase in expression, while 39C6.5, 39C1.5, and 33B7.3 showed a slight increase.

(b)Is there accumulation of mRNA for IL-2 receptor expression after stimulation of B cells with BCGF?

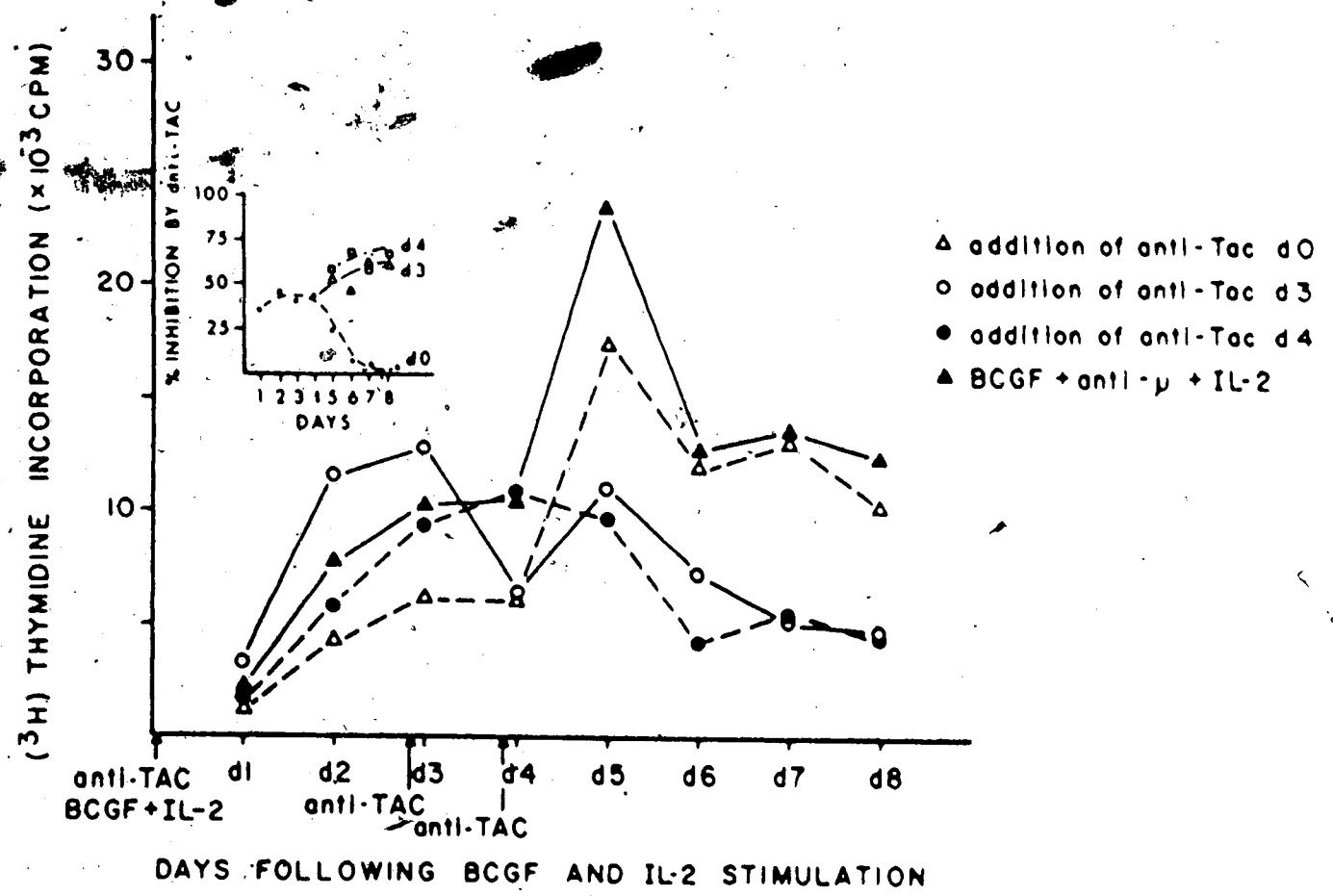

Figure 6. Time course study of the kinetics of inhibition by anti-Tac on the proliferation of B cells stimulated with BCGF and IL-2

B cells ($2x10^5$/well) were cultured in anti-mu-coated microwells after overnight incubation BCGF and IL-2 were added at optimal concentrations. A pool of anti-Tac MAb (33B3.1 and 39C6.5) was added 1:20 dilution to some cultures at the time of adding the factors or 3 days or 4 days later. ^3H thymidine incorporation in parallel cultures was determined every day for 8 days

Table VI. Time course of the kinetics of anti-Tac inhibition of proliferation of B cells stimulated with BCGF and IL-2.

Factors added	[^{3}H] Thymidine Incorporation (cpm) ± SD							
	D1	D2	D3	D4	D5	D6	D7	D8
Anti-mu	1420	2148	1381	382	430	660	840	450
Anti-mu + BCGF	1942	5612	8708	9563	19844	15019	20253	13795
Anti-mu + BCGF + IL-2	2092	7860	10134	10666	23328	12830	13695	12450
Anti-mu + BCGF + IL-2 + anti-Tac D0	1355	4191	6024	6008	17730	12010	13164	10075
Anti-mu + BCGF + IL-2 + anti-Tac D3	3439	11856	12840	6124	11025	7163	5338	4842
Anti-mu + BCGF + IL-2 + anti-Tac D4	1924	5919	9398	10928	9623	4295	5776	4824
Control	880	726	652	368	820	338	872	573

Table VIIa. Comparison of Various Systems for B Cell Stimulation with Respect to the Expression of Related Activation Antigens

	Control	BCGF + IL-2	BCGF + SAC	BCGF	SAC	EBV 55V 8
83H1	91	96	90	94	81	92
7H3	33	60	52	69	45	89
41H16	50	55	46	73	52	32
4F2	42	63	41	73	63	30
5OH19	39	55	-	73	52	25
BK19	24	46	34	61	57	25
IgM	85	46	46	60	87	39
IgG	95	80	81	92	-	-
OKT3	16	15	5	21	24	15
9.6	-	12	6	16	19	14
PBS	10	8	8	11	9	10

The results of this experiment are expressed as % Positive Fluorescent cells at the logarithmic integrated green fluorescence scale

Table VIIb. The RFI Value of the Same Antigens Whereby it Gives a Quantitative Estimate of the Amount of Antigen Present

	Control	BCGF + IL-2	BCGF + SAC	BCGF	SAC
83H1	10.26	12.40	11.43	13.45	10.26
7H3	-	1.08	1.62	4.30	0.95
41H16	3.87	3.47	7.02	7.21	2.25
4F2	1.11	7.02	3.12	7.02	4.80
5OH19	10.83	11.13	-	17.16	10.26
BK19	2.79	4.55	4.43	14.08	4.43
IgM	11.12	1.71	33.80	1.46	7.62
IgG	7.62	9.98	9.70	9.70	-
OKT3	1.00	1.11	0.68	1.05	0.92
9.6	-	1.08	0.72	2.72	0.89
PBS	1.00	1.00	1.00	1.00	1.00

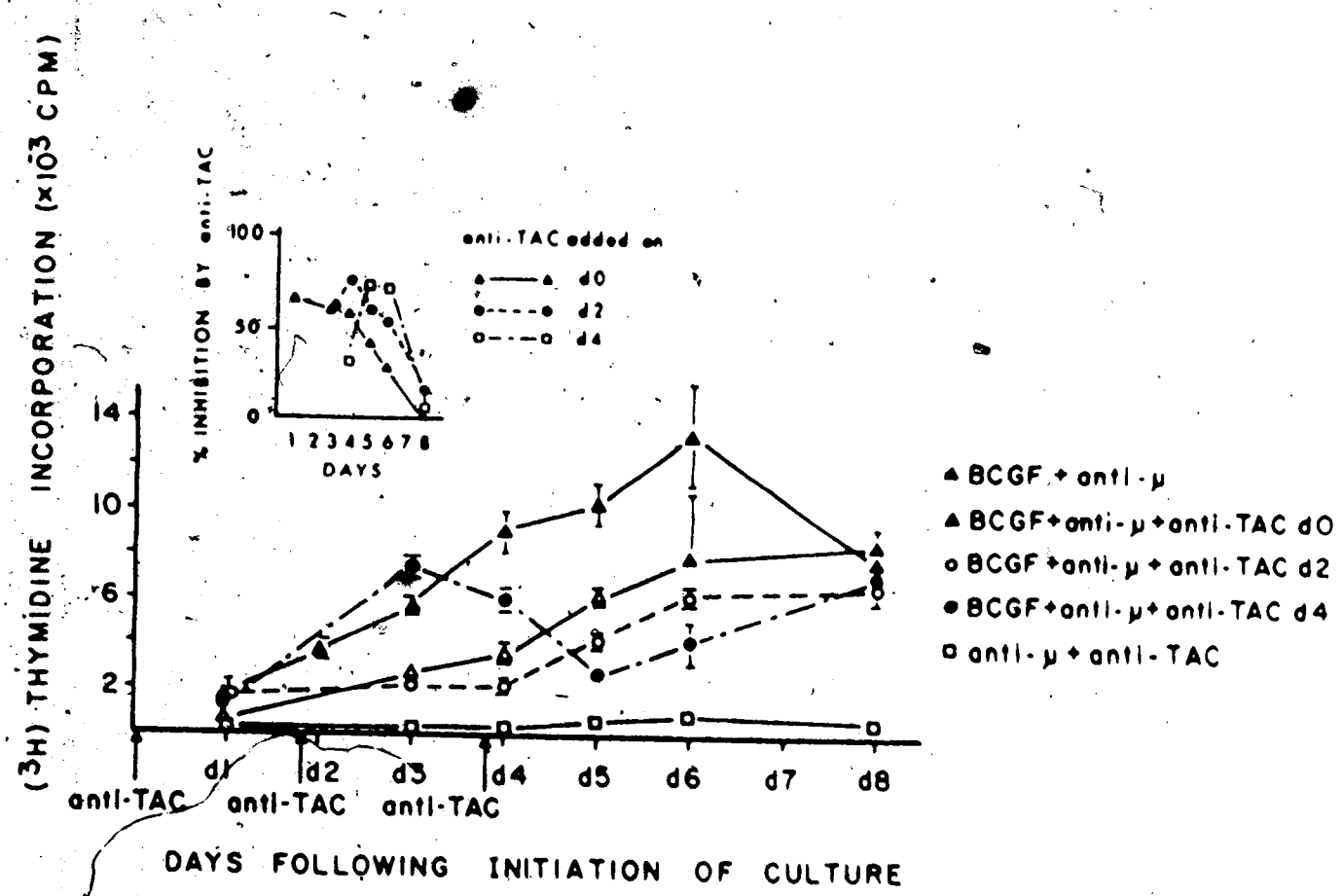

Figure 7. Time course study of inhibition of BCGF induced B cell proliferation with anti-Tac.

B cells ($2x10^5$/well) were cultured in anti-mu-coated microwells. After overnight incubation BCGF was added at 1:10 dilution. A pool of anti-Tac (69.C65 and 33B3.1) was added (1:20 dilution) at the same time as BCGF or 2 days or 4 days later. ^3H thymidine incorporation in parallel cultures was measured every day for 8 days.

Table VIII Selection of Monoclonal Antibodies

Name	Specificity	Source
83H1	anti-class I heavy chain	P. Mannoni
7H3	anti-DR	B.M. Longenecker
41H16	B cell marker	
5OH19	Activated B cell crp 24	
BK19	anti-transferrin receptor	P. Mannoni
4F2	activation antigen	ATCC
33B3.1)	anti-Tac recognizing different epitopes of type V	C. Mawas
39C6.1)		D. Olive
39C1.5)		
33B7.3)		

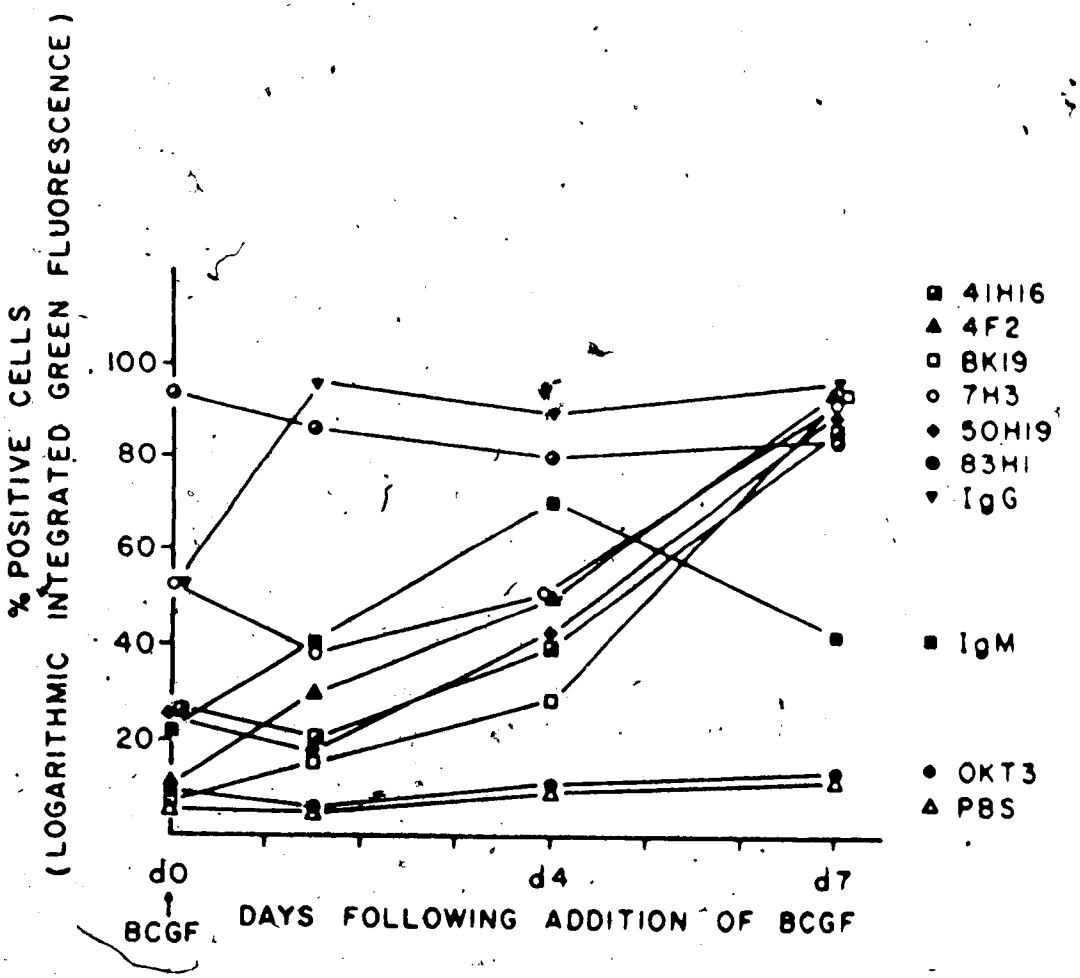

Figure 8a. Kinetic study of dantigen expression on human B cells activated with anti-mu followed by BCGF stimulation.

B cells were cultured (10^6/ml) in 5 cm diameter costar plates coated with anti-mu (25 ug/ml). After 24 hours optimal concentration of BCGF was added to the cultures. At various times after the addition of BCGF the expression of different activation antigens was examined by indirect immunofluorescence and flow cytometry. Non-stimulated and dead cells were gated out on the basis of forward light scatter. 10^4 cells were analyzed and the results expressed on log fluorescence scale.

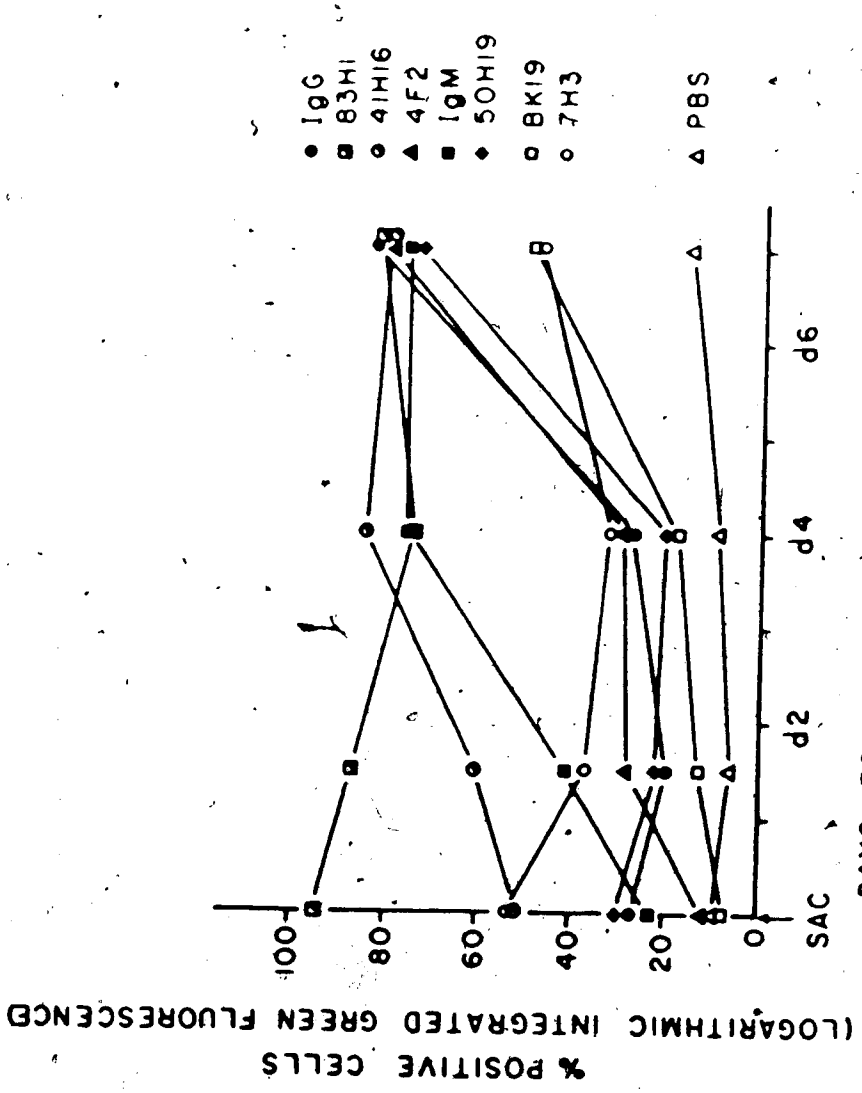

Figure 8b Kinetic study of antigen expression on human B cells activated with anti-mu followed by SAC stimulation

Same experiment as described in 8a was conducted except cells were stimulated with SAC alone at 1:10000 dilution

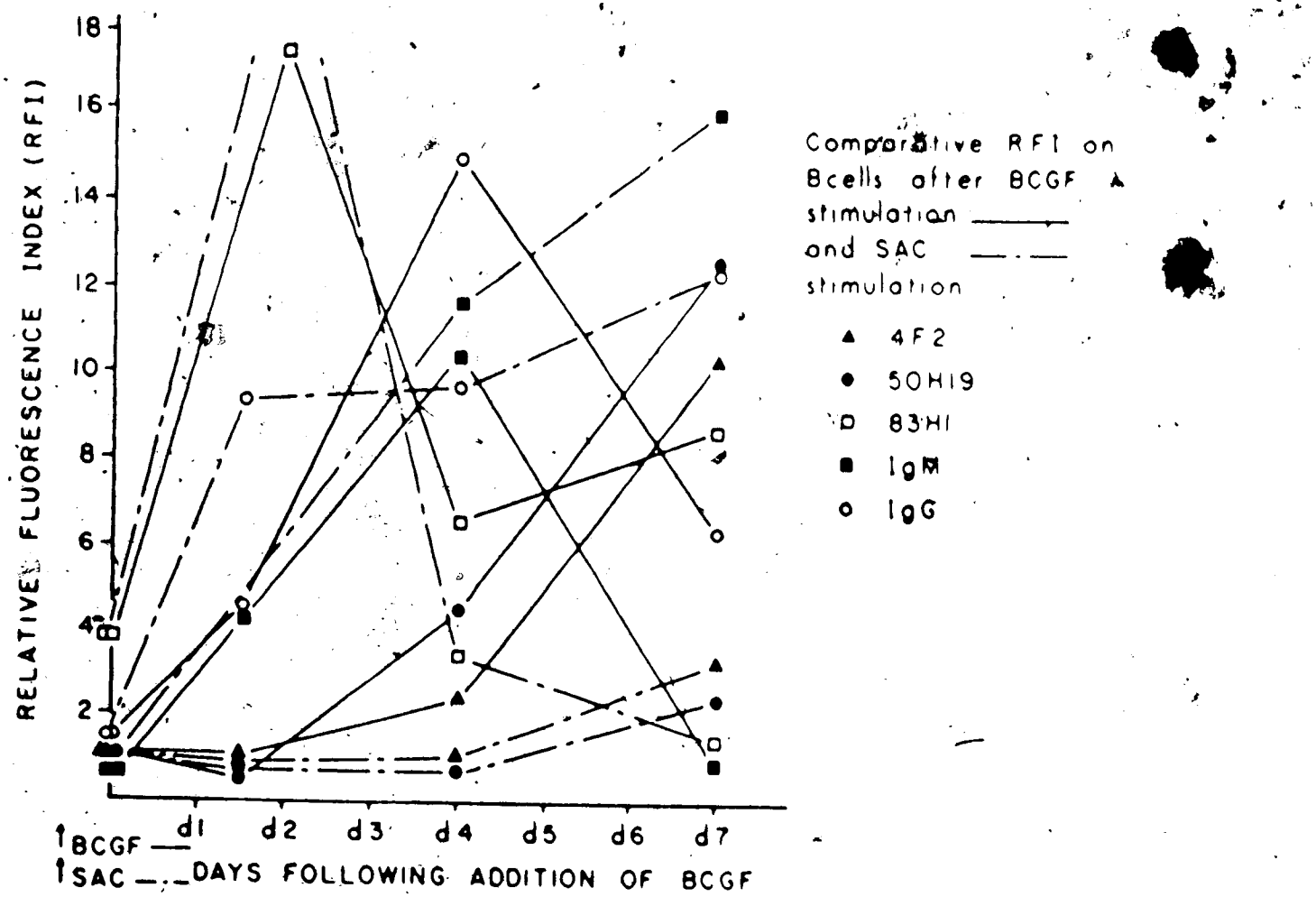

Figure 8c : Comparison of the change with time in the antigen density on human B cells stimulated with BCGF or SAC following stimulation with anti-mu.

Same experiment as in Figures 8a and 8b. The data are expressed as relative fluorescence index

The last part of this project consisted in looking for the transcriptional activity of IL-2 receptor gene occurring in B cell activation. Using cytoplasmic dot hybridization technique by White and Bancroft (1982) it was possible to detect very low levels of mRNA .

(c) Is Tac expression of B cells responsible for the increase of proliferation when given IL-2 and BCGF?

Table VI and Figure 6 summarize the results of inhibition by a pool of anti-Tac MAbs on the proliferation of B cells stimulated with BCGF and IL-2 following activation with anti-mu. Anti-Tac added at day 0 together with the BCGF and IL-2 showed inhibitory effect to be maximum at 47% inhibition by day 2 and it declined by day 7. However, when anti-Tac was added on day 3 or day 4, the inhibition was apparent by 24 h and that inhibition (in contrast to the inhibition noted following addition of anti-Tac on day 0) (up to 67%) did not ultimately recover.

The next question asked was whether anti-Tac inhibits the additive effect of IL-2 on proliferation of B cells or whether it crossreacts with the BCGF receptor. To attempt to answer this question the effect of anti-Tac was studied on BCGF stimulated B cells after giving the activation signal with anti-mu. The results which are shown in Figure 7 and data summarized in Table III show

Table XI. Effect of 4F2 on Proliferation of B Cells by BCGF

Factors Added on Day 0	cpm [^{3}H] Thymidine Incorporation						
	D1	D2	D3	D4	D5	D6	D8
Anti mu	678	550	350	311	324	2128	555
Anti-mu + BCGF	1918	3708	5886	9016	10356	13661	9104
Anti mu + BCGF + 4F2	1350	1410	2940	1742	1509	1048	552
Anti mu + 4F2	776	371	388	556	469	718	445
4F2	679	-	367	332	480	1160	382
Control	-	497	302	340	408	974	335
	% Inhibition						
Anti mu+ BCGF + 4F2	30	62	50	81	85 5	92	94

Table XII. Effect of Anti-transferrin Receptor on BCGF Proliferation Signal on B cells

Factors Added	[^{3}H] Thymidine Incorporation (cpm)							
	DO	D1	D2	D5	D6	D7	D8	D9
Anti-mu	320	850	1522	780	488	1066	1258	714
Anti-mu + BCGF	415	3249	9474	15744	15044	11974	13668	9886
Anti-mu + anti-transferrin DO	920	1812	9885	7207	863	786	988	778
Anti-mu + anti-transferrin R D3	-	-	-	7894	6318	6518	2523	1714
Anti-mu + anti-transferrin R	392	914	938	576	184	179	374	173
Anti-transferrin R	357	530	571	651	584	222	1070	304
Control	244	640	566	464	291	891	1106	1836
	% Inhibition							
Anti-mu + BCGF + anti-transferrin R DO		44	49	64	95	94	93	93
Anti-mu + anti-transferrin R D3		-	-	50	58	46	82	83

Table IX. Comparative Study of the Kinetics of B Cell Antigen Expression Between BCGF or SAC Induced Proliferation of B Cells Activated With Anti-mu

	Resting		BCGF						SAC					
	Day 0		Day 1		Day 4		Day 7		Day 1		Day 4		Day 7	
			%LIGF	RFI	%LIGF	RFI	%LIGF	RFI	%LIGF	RFI	%LIGF	RFI	%LIGF	RFI
83H1	97	3.98	87	17.6	80	6.61	86	8.7	87	0.22	75	3.9	81	1.46
OKT3	9	0.92	7	1.2	11	1.29	14	0.72	8	1.0	9	0.94	26	0.6
9.6	8	0.97	7	1.3	11	1.19	13	0.74	8	1.0	10	1.0	21	0.9
7H3	53	3.66	38	4.9	51	4.55	92	20.7	37	3.1	32	3.79	48	1.02
41H16	27	1.42	20	1.54	41	2.25	86	6.95	21	1.14	28	1.81	84	3.5
IgM	22	0.65	41	4.3	71	14.2	42	0.97	41		76	11.75	46	16.26
IgG	52	1.46	96	4.68	90	15.0	96	6.3	61	9.4	85	9.7	80	12.4
BK19	8	0.65	16	0.94	29	1.0	94	5.2	13	2.92	18	0.37	49	1.5
4F2	11	1.0	29	1.03	50	2.44	94	10.26	29	1.0	30	1.02	80	3.3
50H19	25	1.0	19	0.5	43	4.55	90	12.74	22	0.97	21	4.55	74	2.4
33B3.7	10	0.94	9	0.9	15	0.95	15	0.92	12	1.3	6	0.55	20	0.76
39C65	11	1.02	11	0.87	13	1.08	14	0.95	10	1.3	10	1.08	20	0.85
PBS	9	1.0	8	1.0	10	1.0	8	1.0	6	1.0	9	0.1	16	1.0

that there is an inhibitory effect of anti-Tac on B cells stimulated with BCGF. The pattern of inhibition is different from the previous data shown in Figure 6. It is an ephemaral inhibition of two days, when anti-Tac is added at day 2 while the same pattern of inhibition (Figure 6) is observed when added at day 0.

III RELATIONSHIP BETWEEN ANTIGENS AND B CELL ACTIVATION AND PROLIFERATION.

For that purpose a comparative investigation was done using different stimuli for B cell activation as well as different B cell lines at different stages of differentiation. The main purpose of this phase was to use different modes of stimulations to define the kinetics, and the amplitude of cell growth as well as the variation of the expression of some surface antigens and hence to select the most appropriate ones for defining the activation state of B cells.

The first model considered for B cell activation was the EBV transformed human B cell lines, a model established in our laboratory for the production of clinically relevant human monoclonal antibodies, Winger et al., (1983).

Comparative studies between the different modes of activation (Tables VIIa and VIIb) showed differences with respect to the density of the expression for a given

antigen following stimulation. Nevertheless, the mode of action for any of these inducers remains unanswered. However, the density of DR antigen (7H.3) was selected as a control for the best system of B cell activation. B cells which were previously activated with anti-mu expressed the highest density of DR (Table VIb) when given BCGF. The same was true with respect to 50H19, 4F2, and BK19 expression. In contrast, when these same B cells were given IL-2 in addition to BCGF, even though the ^{3}H thymidine uptake was higher, IL-2 seemed to suppress the increase in density of DR antigen as well as 50H19 and 41H16 while having no effect on 4F2 antigen density. The anti-transferrin receptor (BK19) in contrast, presented some increase in density showing that all the cells were metabolically active throughout the different systems of stimuli for the induction of B cell proliferation.

As a conclusion, proliferation correlates with the expression of some activation antigen by virtue of the increase of their expression on the surface of B cells stimulated with either BCGF or BCGF and IL-2 or BCGF and SAC. In the next section I have examined this question i.e., namely the role of 4F2 and BK19 in the induction of proliferation of B cells induced with BCGF alone after activation with anti-mu. Eventually, as illustrated in Figure 9 and summarized in Table XI, 4F2 inhibits the BCGF

induced proliferation of B cells, starting at the onset of the signal while BK19 inhibited late in the process. Neither of the MAbs induced proliferation by themselves. Control experiments were run with an unrelated MAb of the same isotype IgG_{2a} but it did not have any effect on the proliferation profile of B cells induced by BCGF.

Hence, 4F2 cannot recognize an epitope in common with BCGF receptor. However, it plays an important role in the processing of the proliferation signal by BCGF. The last report on the function of the 4F2 molecule on the surface of cells is that of Na^+/Ca^{2+} pump exchanger (personal communication by Dr. Michelle Letarte), Michalak, et al. (1985).

Thus, 4F2 has an inhibitory effect of 50% and more starting day 2 after its addition into BCGF stimulated B cells, the anti-transferrin receptor MAb BK19 shows an inhibitory effect of 50% more starting day 5 after its addition in the same system of B cell proliferation, as shown in Figure 9 and Table XI.

The second question asked in the experiment illustrated in Figure 2, was how does the profile of the expression of activation antigens correlate with the proliferation signal?

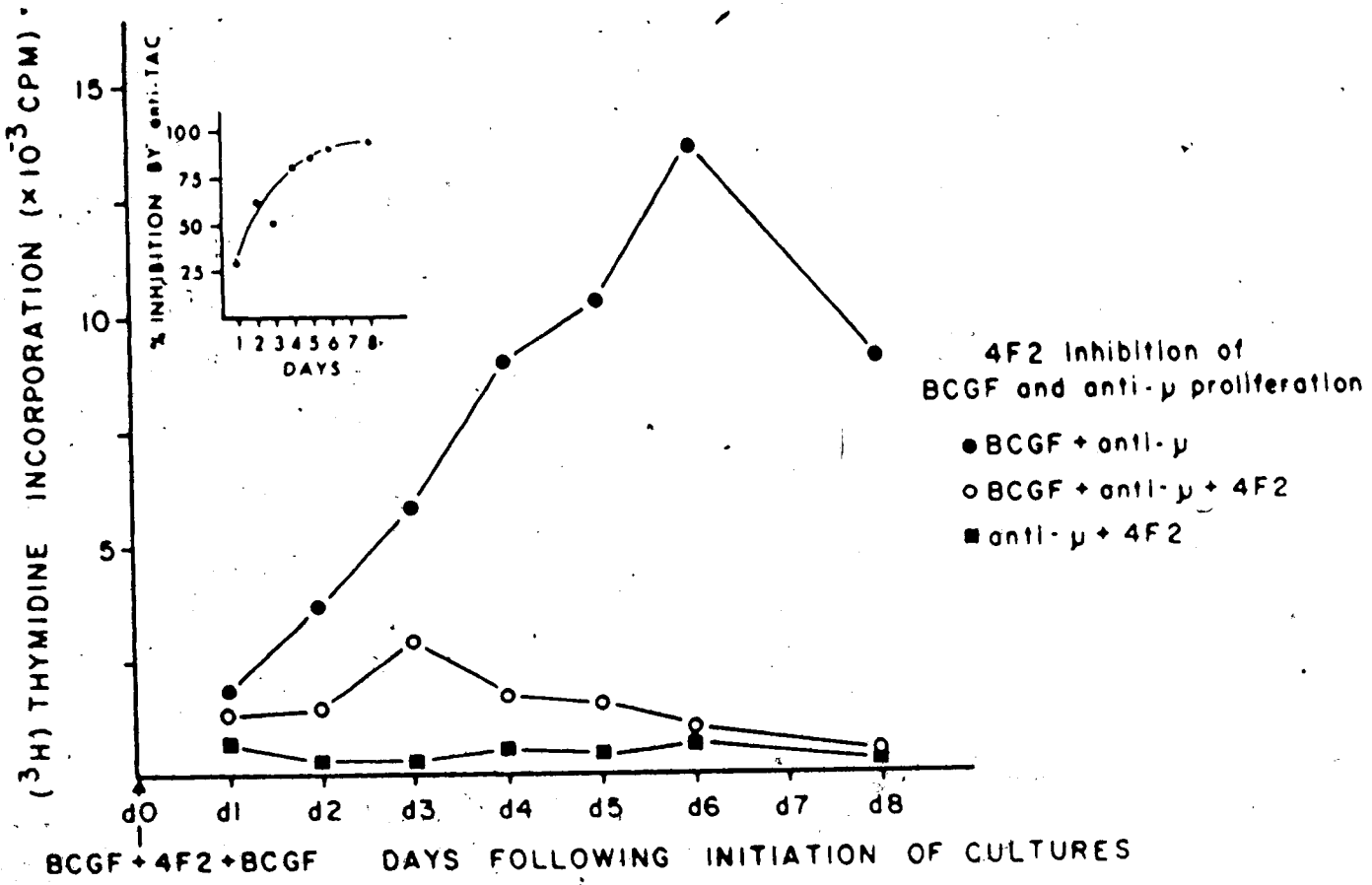

Figure 9. Time course study of the inhibition of B cell proliferation (stimulated with BCGF and anti-mu) by 4F2 MAb (1:500)

Human B cells (2×10^5 well) were added in anti-mu-coated microwells. After overnight incubation, BCGF was added in 1:10 dilution alone or with 4F2 MAb (1:500 dilution of ascites). ^3H thymidine incorporation was measured at various times.

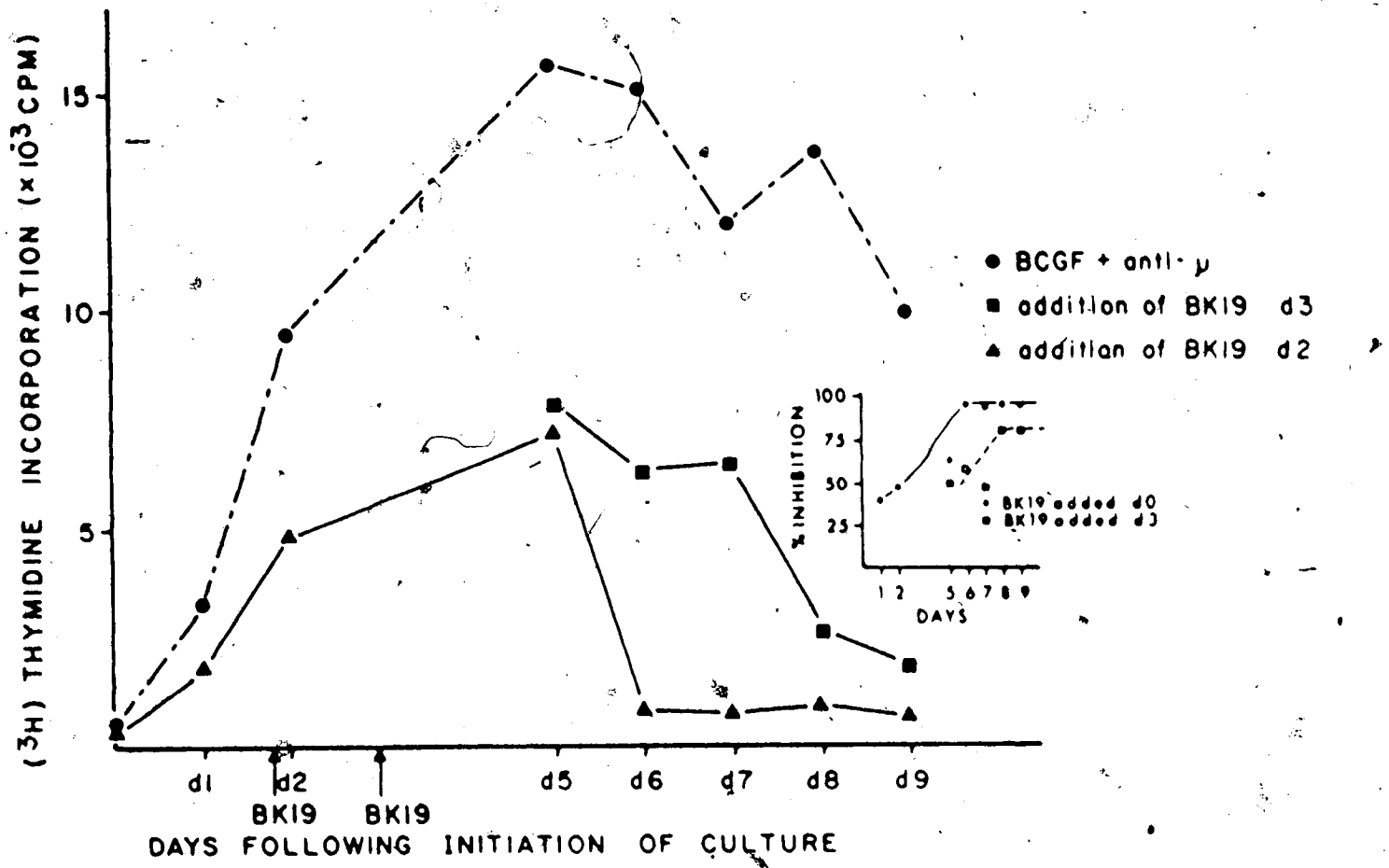

Figure 10. Kinetics of inhibition of proliferation of B cells by anti-transferrin receptor.

Human B cells ($2x10^5$/well) were added to anti-mu-coated microwells. After overnight incubation BCGF was added to all the cultures in 1:10 dilution. To some cultures anti-transferrin receptor MAb BK19 from hybridoma supernatant was also added (1:10 dilution) either at the same time as the factor or 3 days later. ^3H thymidine incorporation was measured at various times.

Table X. Kinetics of the Variation of Expression of Particular Antigens Available from the Human Leukocyte Workshop. The Results Represent % Fluorescent Positive cells.

	D0	D1	D2	D4	D6	D8
B1	50	52	67	86	83	89
B4	52	61	19	34	20	29
B5	40	66	-	39	24	29
B11	0.6	8	14	24	18	28
B14	12	25	47	35	21	31
B19	54	7	14	21	16	25
B22	-	69	65	42	24	28
B28	39	41	57	38	24	29
B29	10	14	41	45	44	49
B17	-	56	34	44	54	31
B24	-	68	65	43	22	27
B34	-	24	56	39	22	33
B43	30	39	54	41	23	30
B39	0.7	9	13	23	20	25
BK19	12	11	35	63	74	85
7H3	47	92	86	98	98	99
M18	95	97	98	99	98	99
4IH16	52	57	54	71	64	97
5OH19	19	26	56	54	54	93
4F2	28	45	53	82	86	99
IgM	33	-	-	-	-	94
IgG	2	13	34	12	88	69
LII2	63	82	81	94	92	98
10G33	6	7	13	21	26	29
39C6.5	1	5	14	27	14	36
33B3.1	2	5	12	25	16	36
39C1.5	1	6	9	22	19	33
33B3.7	2	5	14	21	18	33
PBS	0.7	2	10	15	13	28

In order to answer this question a comparitive study was carried out on the expression of activation antigens on B cells induced to proliferate with either BCGF or SAC. The MAb used in this study are listed in Table VIII. These two signals showed tremendous differences with respect to proliferation and these differences were reflected in the expression of some of the activation antigens which may play an important role in B cell proliferation (Figures 8a, b, c, and d are summarized in Table IX). The antigen density on these cells is expressed as the Relative Fluourescence Index (RFI) which is defined in Materials and Methods . Comparing the RFI of various antigens between BCGF and SAC stimulated B cells it is clear that BCGF induced, in parrallel to proliferation, an increase of DR, 41H16, BK19, 4F2 and 50H19. Of particular interest is the finding that the density of these antigens increased even when proliferation ceased. As a conclusion, when B cells are stimulated with BCGF the maximum density of activation antigens correlated with post proliferation events on day 5 as shown for 4F2 and 50H 19 in Figure 3c.

Stimulation of B cells with SAC, on the otherhand, induced a differential expression of antigens. There was a slight increase in the expression of 41H16 and 4F2 during stimulation whilst that of 7H3 and BK19 decreased during this time. In contrast, 50H19 expression increased peaking on day 4 and decreased by day 7..

Table X, in contrast, illustrates that the expression of some antigens increased between day 2 and 4 and then decreased as proliferation goes on. I did not investigate further the expression of those antigens.

DISCUSSION

The two general questions tackled in this thesis were:

a) Is IL-2 inducing B cell proliferation by itself or in association with other signals, like anti-mu and BCGF?

b) Is the presence of certain activation antigens associated with B cell activation and proliferation?

The first question asked in this system was, whether there is cooperation between BCGF, and IL-2 in stimulating proliferation?

If cooperation between the two factors exists, synergy between these proliferative actions would be predicted. This has been demonstrated in Figure 3 and Table III. Significantly, IL-2 alone has almost no stimulatory effect in the proliferation of B cells. In the same experiment the observation that anti-Tac inhibited proliferation associated with IL-2 and BCGF led to the hypothesis that BCGF stimulates expression of the IL-2 receptor as a first requirement for IL-2 responsiveness. Subsequently, a sequence of events were hypothesized, i.e., the BCGF signal would trigger events leading to gene activation for the IL-2 receptor resulting in accumulation of IL-2 receptor mRNA and in turn the expression of the IL-2 receptor on the surface of B cells.

The second step was to show evidence for the IL-2 receptor expression on the surface of B cells stimulated with BCGF; time course study of the expression of IL-2 receptor was followed using flow cytometry analysis. Therefore the effects of BCGF and of BCGF plus IL-2 on Tac expression were compared. The results in Figure 5 and Table V clearly demonstrate that BCGF induced Tac expression on B cells. Furthermore, the addition of IL-2 neither increased nor decreased Tac expression, suggesting that in the case of B cells IL-2 had no additional effect on upregulating IL-2 receptor expression. Further control experiments would have been to look for the IL-2 receptor expression with B cells stimulated with IL-2 alone. However based on the lack of proliferation in the presence of IL-2 I would predict that these would not express the IL-2 receptor.

I have shown, as have others, that stimulation of B cells by SAC and BCGF strongly stimulate both Tac expression and cell proliferation. However, the peak of proliferation with BCGF and SAC is delayed by one day over the peak of proliferation induced by BCGF or BCGF and IL-2. The addition of IL-2 to BCGF and SAC delays the peak of proliferation by a further 24 hours as illustrated in Figure 2. This shows that IL-2 has different effects on B

cell proliferation when stimulated with BCGF or BCGF and SAC. However, I have not determined whether anti-Tac antibody would block the BCGF and SAC stimulation.

In my first attempts to demonstrate the accumulation of IL-2 receptor mRNA in the cytoplasm after BCGF stimulation I used the cytodot blotting technique. In this experiment I obtained only a weak signal suggesting the presence of IL-2 receptor mRNA. I subsequently turned to northern blot analysis which would demonstrate specificity in the hybridization reaction. However my attempts at this technique failed. Dr Kehrl at N.I.H., Bethesda U.S.A., has shown evidence for accumulation of mRNA for the IL-2 receptor in B cells stimulated with phorbol ester (PMA) and with SAC (personal communication). They have shown two transcripts, yet, no one has observed this in B cells stimulated with BCGF after anti-mu activation.

In conclusion, Tac is expressed on B cells following BCGF stimulation. Whether distinct B cell subsets are involved here can be determined by doing two or three color FACS analysis.

Having shown that Tac is expressed on B cells stimulated by BCGF and anti mu I then examined the possible mechanistic roles it could play in B cell function by asking the following question:

Is the synergistic effect observed between BCGF and IL-2 in stimulating B cell proliferation mediated through the IL-2 receptor or are there separate receptors for the two lymphokines?

If the IL-2 receptor is involved I would expect the addition of anti-Tac to the cultures to inhibit B cell proliferation. Indeed, it was found that anti-Tac did inhibit B cell proliferation. In the cultures containing BCGF and IL-2, the greatest degree of inhibition was observed when anti-Tac was added late to the cultures, as shown in Figure 6 and Table VI. This result is consistent with a role for the IL-2 receptor in B cell proliferation, and furthermore, it is required at late stage of the response. This correlates with the finding that it takes 3 days for Tac expression on stimulated B cells. In contrast in cultures containing BCGF alone there was no long lasting inhibition when anti-Tac was added late to the cultures. As in the previous example the addition of anti-Tac at time zero resulted in transient inhibition of proliferation. This short term nature of the inhibition could be due to shedding and/or secretion of IL-2 receptor from the surface of B cells. Evidence of a soluble form of IL-2 receptor was recently shown by Rubin et al., (1985). The finding that anti-Tac did not augment B cell proliferation is consistent with similar findings in the T cell system, whereas in

other biological systems anti receptor antibodies can mimic the specific ligand e.g. the anti-insulin or anti idiotype antibodies.

The nature of the detected Tac antigen on B cells is not clear. The antigen could mediate high affinity or low affinity IL-2 binding or it perhaps might even bind BCGF. Preliminary results (Figure 7) show that anti-Tac inhibits proliferation of B cells stimulated by BCGF alone. These results suggest either involvement of IL-2 elaboration as a result of anti-mu and BCGF treatments or shared Tac epitopes on separate IL-2 and BCGF receptors. In support of the latter hypothesis comes from the recent literature: Leonard et al., (1985) have shown that the exons 2 and 4 of the structure of the gene encoding the IL-2 receptor showed homology with the recognition domain of human complement factor B. The resolution of this problem awaits isolation of the BCGF receptor.

The second question which I addressed was whether activation antigens besides the IL-2 receptor play a role in the induction of B cell proliferation by BCGF. As illustrated in Figures 9 and Table XI the MAb 4F2 inhibits BCGF induced B cell proliferation. Again, this MAb on its own was not stimulatory.

There is evidence that 4F2, which was first described by Eisenbarth et al., (1980) recognizes a cell surface molecule that might play an important role in human lymphocyte responses. This MAb can both enhance or suppress human T cell and B cell function in vitro depending on the stimulus employed,(Haynes et al., 1981: Gerrard et al., 1984).

Little is known about the function of 4F2. It is also found on non lymphoid cells and a recent report by Michalak et al., (1985) suggests that it recognizes an epitope on the Na^{+}/Ca^{2+} pump exchanger, on cardiac cells.

In addition to 4F2, MAb to the transferrin receptor (BK19) also inhibited B cell proliferation when added to cultures on day 0 or day 3 (Figure 10). This finding is consistent with previous observations and those reported in this thesis, that IL-2 and BCGF induce transferrin receptor expression (results part II.). Because the epitopes recognized by 4F2 and BK19 are also present on non lymphoid cells it argues that once cells receive their "tissue specific signal" they use similar if not identical secondary machinery to proliferate and differentiate.

Experiments were also conducted to determine whether any of these antigens were expressed in a cell cycle dependent manner on BCGF stimulated B cells. This was determined by the simultaneous staining of cells for DNA

content with propidium iodide and with the various MAbs. In summary, there was no evidence that any of these antigens showed a cell cycle dependence (data not presented) i.e. regardless of the DNA content of the cells the amount of antigen expressed was the same.

As a conclusion, these complex molecules which increase in their expression upon B cell stimulation might play an important role in B cell proliferation. If BCGF requires internalization this could be mediated by certain activation antigens Pernis (1985). Still, there can be no definitive answer to the understanding of the mechanism of action of BCGF as long as there is no information about the molecular genetics of BCGF or its receptor.

As a general conclusion B cell proliferation is a complex mechanism, and the heterogeneity of B cell populations suggests that different subpopulations of B cells defined by different stages of development within a single lineage have requirements for distinct lymphokines that regulate their growth. In B cell proliferation, BCGF synergized with IL2 giving a maximum response accompanied with an increase in the expression of surface antigens such as 4F2, DR, transferrin, 50H19 and IL-2 receptor most of which are cell cycle dependent.

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