Induction of B Cell Apoptosis by TH0, but not TH2, CD4$^+$ T Cells

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Abstract

Engagement of the T cell receptor molecules with MHC-antigen complexes presented by B cells ascertains antigen specificity in T cell-dependent help. Ligation of MHC molecules on the surface of B cells, however, has not only been implicated in antigen-specific T-B cell interaction, but has also been linked to the induction of B cell apoptosis. To examine the role of T helper cells in either induction of immunoglobulin synthesis or B cell apoptotic death, we have facilitated T cell receptor-MHC interaction through a bacterial superantigen. CD4$^+$ T cell clones could be categorized into two clearly distinct subsets based upon their ability to promote B cell help in the presence of superantigen. One subset of T cell clones supported immunoglobulin synthesis, and thus functioned as effective helper cells. B cells interacting with the second subset of T cells did not differentiate into antibody-secreting cells, but underwent apoptosis. Both types of helper cells were able to provide contact help after anti-CD3 stimulation. Induction of apoptosis was a dominant phenomenon; the addition of the superantigen suppressed immunoglobulin production in B cells activated by anti-CD3-stimulated helper T cells, indicating that the T cells delivered an apoptotic signal to the B cell. T cell clones providing effective MHC restrictive B cell help could be distinguished from T cells facilitating B cell apoptosis based on their lymphokine secretion profile. Induction of B cell apoptosis was a feature of T cells with a TH0 lymphokine pattern. Promotion of MHC-restricted B cell help was associated with a TH2 lymphokine profile. TH1-derived cytokines alone could not substitute for apoptosis-inducing T cells. (*J. Clin. Invest.* 1995. 95:564-570.) Key words: MHC molecules • T-B cell interaction • autoimmunity • tolerance

Introduction

T cell-dependent B cell activation is a multistep process involving contact help and the action of T cell-derived cytokines (1). Contact help is mediated by a number of molecular interactions between cell membrane proteins expressed on T and B cells (2). One receptor ligand pair which appears to have a crucial contribution in T-B cell interaction is the CD40 molecule pairing with its ligand (2-4). CD40 is constitutively expressed on B cells and B cells can be stimulated to proliferate and differentiate in the presence of immobilized anti-CD40 antibodies (5-8). The CD40 ligand is transiently expressed on helper T cells and participates in contact help (9-11). Contact help per se is antigen nonspecific. Specificity of the antibody response is maintained by the antigen-specific stimulation of the T cell required for CD40 ligand expression. B cells can process and present antigen to the T cell and, therefore, induce T cell activation. In the current paradigm, B cell recognition of an antigen by surface immunoglobulin does not deliver a signal that is required for T cell-dependent B cell activation. Thus, the recognition of a processed antigen presented in the context of MHC class II molecules on the B cell is the only T-B cell interaction that introduces antigen specificity and ensures the specificity of the antibody response (12).

The MHC class II molecules on B cells not only function as antigen-presenting restriction elements, but also represent signal transducing receptors. It has been demonstrated that ligation of MHC class II molecules on B cells results in elevated levels of intracellular cAMP and inhibits subsequent responses to mitogenic signals (13, 14). Interestingly, an increase in intracellular cAMP induced by either pharmacological agents or by cross-linking MHC class II molecules induces apoptosis in resting B cells (14). The physiological relevance of this mechanism in the interaction of human T and B cells has not yet been demonstrated. In particular, induction of B cell apoptosis by signaling through MHC class II molecules raises the question whether the engagement of T cell receptors (TCR)[1] with MHC molecules can result in B cell death. Essentially, interaction of T and B cells via TCR recognizing the MHC antigen complex may have one of two very different outcomes, B cell activation or B cell apoptosis.

To investigate whether a dichotomy exists for MHC-restricted T-B cell interaction, we have analyzed a series of human CD4$^+$ helper T cell clones for their capability to induce either immunoglobulin production or B cell apoptosis. TCR-HLA-DR interaction was facilitated by a bacterial superantigen, staphylococcal enterotoxin D (SE D). We were able to define T cell clones that served as excellent helpers, whereas a second subset of T cell clones failed to provide help for B cell differentiation. Both types of helper T cells promoted immunoglobulin production when stimulated through cross-linking with anti-CD3 antibodies and help was provided independently of recognition of MHC antigen complexes on the surface of the B cells. The subgroup of T cell clones failing to promote immunoglobulin synthesis in MHC-restricted interaction did indeed induce B cell apoptosis. T helper cell clones with and without the ability to induce apoptosis could be distinguished based on their lymphokine secretion pattern. Apoptosis inducing helper cell clones were characterized by the coproduction of IL-2, IFN-γ, and IL-4, whereas T cells providing MHC-mediated help exhibited a TH2-like pattern. These data suggest that the activation of resting B cells in an antigen-specific MHC-restricted

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Received for publication 18 April 1994 and in revised form 12 August 1994.

1. *Abbreviations used in this paper: SE D, staphyloccal enterotoxin; TCR, T cell receptor.*

interaction is strictly controlled by the functional commitment of the interacting T cells.

Methods

Human T cell clones. 5×10^5 PBMC/ml from a normal donor were cultured in culture plates coated with anti-CD3 mAb (OKT3, 50 μg/ml). After 24 h, T cells were cloned at a concentration of 0.3 cells/well in the presence of 3×10^4 cells/well irradiated filler cells and 20 U/ml rIL-2 (Cetus Corp., Emeryville, CA). $CD4^+$ $CD8^-$ T cell clones were identified by FACS® analysis and were selected for these experiments. The clones were maintained in culture by stimulation every 7–10 d in the presence of exogenous IL-2.

Proliferative response to the bacterial superantigen SE D. Triplicates of 3×10^4 cloned T cells were cocultured with 4×10^4 irradiated antigen presenting cells (lymphoblastoid B cell lines [LCL]) in the presence of 0.5 μg/ml SE D (Toxin Technology, Sarasota, FL) for 48 h. Proliferative responses were assessed by [^{3}H]thymidine incorporation. Control microcultures included T cell clones cultured with antigen presenting cells only and T cell clones stimulated with immobilized anti-CD3. Specific proliferation was calculated by subtracting background proliferation from induced proliferation. Results are given as mean counts per minute. Background proliferation of T cell clones cultured with irradiated LCL alone was < 3,500 cpm. Only the SE D–responsive T cell clones were selected for further studies.

T-B cell cocultures. 1×10^5 $CD4^+$ T cell clones were cocultured with 1×10^4 purified B cells in 96 well flat bottom tissue culture plates in the presence of SE D or immobilized anti-CD3 mAb. B cells were purified from PBMC of five normal donors as previously described (15). In brief, 5×10^6 PBMC was incubated with 50 mM L-leucine methylester HCl (Sigma Chemical Co., St Louis, MO) for 45 min at room temperature to deplete monocytes (16). Subsequently, T cells were depleted by rosetting with 2-AET (Sigma Chemical Co.) treated sheep red blood cells. Nonrosetting cells were analyzed by FACS® with antibodies for CD3, CD14, and CD19. The cell population contained < 1% $CD14^+$ and < 5% $CD3^+$ cells. Supernatants of the T–B cell cocultures were collected after 14 d. The concentration of total IgM was determined by ELISA as recently described (15).

L-leucine methylester induces apoptosis in cells which contain lysosomes. To exclude the possibility that the leucine methylester renders B cells susceptible to apoptosis, an alternative method to semipurify B cells was used. PBMC were incubated on plastic dishes for 2 h. Nonadherent cells were rosetted with sheep red blood cells. The B cell–enriched nonrosetting cells were cocultured with T cell clones as described above.

In selected experiments, T–B cell cocultures were performed in the presence of both immobilized anti-CD3 and SE D. The microcultures were started in the presence of immobilized anti-CD3, and SE D was added at the times indicated.

To analyze the influence of cytokines in the T–B cell cultures, T cell clones were cocultured with B cells in the presence of 0.5 μg/ml SE D and the following cytokines: 10–1,000 U/ml in TNF-α (R & D Systems, Inc., Minneapolis, MN), 10–1,000 U/ml IFN-γ (Endogen, Inc., Boston, MA), 10–100 U/ml IL-2 (Cetus Corp.), 2–10 U/ml IL-4 (Schering Corp., Kenilworth, NJ), or 100% supernatant from stimulated TH0 or TH2 clones. To analyze the influence of cell–cell contact, T cell clones were activated with anti-CD3 for 12 h, fixed with 2% formaldehyde, and then cocultured with purified B cells in the absence and presence of SE D.

Multiparameter flowcytometric assay for apoptosis. T-B cell cultures were harvested after 36–40 h and cells were stained with a mixture of FITC-labeled anti-CD19 and anti-CD20 antibodies (Becton Dickinson Immunocytometry Systems, Mountain View, CA) at 4°C for 30 min. After washing, cells were incubated in 2 ml of PBS containing 10 μg/ml lysolecithin and 1% paraformaldehyde at 4°C for 25 min. After washing, cells were treated with 200 μl of 1 mg/ml RNAse and stained with 50 μg/ml propidium iodide for 30 min at 4°C (17). Cells were analyzed on a FACSVantage® (Becton Dickinson Immunocytometry Systems).

Table I. Proliferative Responses of $CD4^+$ T Cell Clones to SE D

Clone	Proliferation (counts)	
	SE D	Anti-CD3
T02	43,557	29,908
T37	47,590	59,244
T25	10,745	7,358
T72	91,853	82,086
T32	7,622	23,055
T08	125,086	42,410
T23	97,071	50,877
T15	80,150	91,065
T04	13,548	30,043
T38	7,360	39,597

Lymphokine production. 1×10^5 cloned T cells were stimulated with immobilized anti-CD3 mAb in flat bottom 96-well microculture plates. Supernatants were harvested after 24 h. To determine IL-2 activity in the supernatants, 8×10^3 HT2 cells/well were cultured in the presence of serial dilutions of culture supernatants. Proliferation was determined by [^{3}H]thymidine incorporation after 24 h. IL-4 activity was determined in a bioassay utilizing its ability to induce the expression of CD23 on a human B cell line, Ramos cells (18). 2×10^4 Ramos cells/well were cultured in the presence of supernatant dilutions and immobilized anti-IgM antibodies. The cells were harvested after 48 h, stained with phycoerythrin-labeled anti-CD23 mAb (Becton Dickinson Immunocytometry Systems), and analyzed by FACS® analysis. Recombinant IL-2 (Cetus Corp.) and recombinant IL-4 (Schering Corp.) were used to standardize the bioassays. IFN-γ was determined in an immunoassay by using commercially available ELISA plates (BioSource International, Inc., Camarillo, CA). Lymphokine patterns for the different T cell clones were confirmed by reverse transcriptase-PCR with primer sets specific for IL-2, IL-4, IL-5, IL-10, and IFN-γ as described (19).

Results

Heterogeneity of T helper cell clones providing MHC-restricted help to B cells. $CD4^+$ T cell clones were established from peripheral blood of normal donors. 10 different T cell clones were selected, which proliferated in response to SE D presented by irradiated lymphoblastoid cells (Table I). None of the T cell clones showed alloreactivity to the lymphoblastoid cell line (data not shown). All T cell clones were tested for their ability to provide B cell help in a 14-d T–B cell coculture system. As shown in Fig. 1, 5 of the 10 T cell clones promoted B cell activation and production of IgM. Concentrations of IgM secreted in SE D–driven cultures ranged from 2.8 to 7.5 μg/ml. T cell clones T04, T08, T15, T23, and T38 failed to provide B cell help. To examine the possibility that the five T cell clones unable to support IgM production had an intrinsic defect and therefore could not serve as helper cells, all T cell clones were tested for their helper capabilities in an antigen-nonspecific contact help system. T cells were stimulated with immobilized anti-CD3 and cocultured with peripheral B cells for 14 d (Fig. 1). All T cell clones were able to induce B cell differentiation and IgM secretion. The ability to provide B cell help differed for the individual T cell clones, IgM production varied between 5.4 μg/ml and 33 μg/ml. In general, lower concentrations of IgM

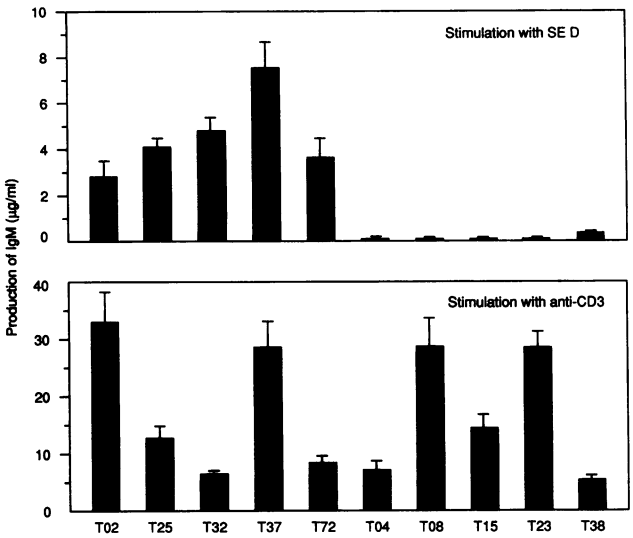

Figure 1. Heterogeneity of T-helper cell clones providing MHC restricted help to B cells. 1×10^5 SE D reactive (as shown in Table I) T cell clones were cocultured with 1×10^4 purified B cells from one donor in the presence of SE D (*top*) or immobilized anti-CD3 (*bottom*) for 14 d. All microcultures were set up in triplicates. The amount of IgM in the supernatant was measured by ELISA. Results of one representative experiment are shown as mean ± SD. Although all of the T cell clones were excellent helpers in the anti-CD3–driven cultures, only half of them provided MHC-restricted help in the presence of SE D. This pattern was reproducible in experiments using B cells from three different donors (data not shown). B cells cocultured with the appropriate T cell clone in the absence of additional T cell stimulatory signals produced < 0.25 μg/ml IgM (data not shown).

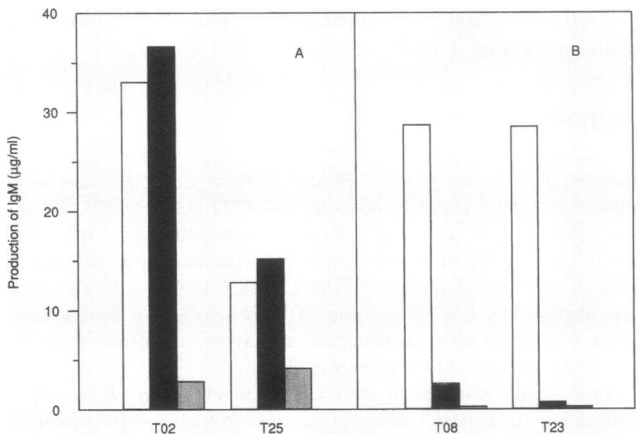

Figure 2. Cross-linking of HLA-DR molecules and TCR molecules inhibits B cell activation. T cell clones T02 and T25 were able to provide MHC-mediated help, whereas clones T08 and T23 failed to do so (Fig. 1). To test the question of whether clones T08 and T23 actively inhibited B cell differentiation, T–B cell cultures were stimulated with a combination of anti-CD3 and SE D. The addition of SE D to anti-CD3–stimulated T–B cell cultures of T cell clones T08 and T23 inhibited IgM production, while it did not influence the helper activity of clones T02 and T25. (□), anti-CD3; (■), anti-CD3 + SE D; (▨), SE D.

were synthesized in superantigen-driven cultures compared to anti-CD3–stimulated cultures. There was no clear relationship in the response pattern to the two distinct modes of activation. T cell clones such as T08 and T23, which were very effective helper cells after anti-CD3 stimulation, failed to induce IgM production in the presence of SE D. T cell clones T32 and T72 effectively promoted B cell help in SE D–driven cultures, but were relatively weak helper cells when activated with anti-CD3. These data suggested that the failure of some T cell clones to induce IgM production when SE D was used to cross-link TCR and MHC molecules was not a general lack of the ability to provide B cell help. Rather, the heterogeneity among T cell clones to function as helpers in the presence of SE D raised the possibility that TCR-HLA-DR cross-linking did not necessarily result in B cell stimulation.

Cross-linking of HLA-DR molecules and TCR molecules of selected T cell clones inhibits B cell activation. Failure to promote B cell help by one subset of T cell clones stimulated with SE D suggests as one possibility, that these T cells were insufficiently activated to provide help. It could also be hypothesized that cross-linking of TCR and HLA-DR molecules provided a negative signal to the B cells and that only certain T cell clones could rescue the B cells from this negative signal. To address these possibilities, we cocultured T cells and B cells in the presence of both SE D and immobilized anti-CD3. The addition of the superantigen to the anti-CD3–driven cultures did not significantly influence the IgM production induced by T cell clones T02 and T25, which were able to provide help to peripheral B cells in the SE D–driven system (Fig. 2). In contrast, T cell clones T08 and T23 did not activate peripheral B cells to produce IgM in the presence of the superantigen (Fig. 1). For these two clones, the addition of SE D to anti-CD3–stimulated T-B cell cocultures resulted in a > 90% inhibition of IgM production (Fig. 2). Similar suppressive effects of SE D on B cell activation and IgM production were found for all T cell clones which proliferated in response to SE D, but could not provide B cell help in the SE D–driven cultures. These results suggested that the cross-linking of the TCR of selected T cell clones and HLA-DR molecules on B cells delivered a negative signal which inhibited B cell activation.

Cross-linking of TCR and MHC by SE D induces B cell apoptosis. To analyze the mechanism of the suppressive action of SE D, we determined the recovery of B cells in T–B cell cocultures by FACS® analysis. These experiments demonstrated that the number of B cells declined in the SE D–driven cultures if T cell clone T08 or T23 were present, but remained stable if T cell clone T02 or T25 were included. The reduction in viable B cells in the presence of T cell clones T08 or T23 occurred between days 2 and 7 of culture. During the first 24 h, the number of B cells remained unchanged, at 4 d the number had declined by 50%, and after 7 d B cells had disappeared from the cultures (data not shown). We, therefore, wanted to address the possibility that the SE D–mediated cross-linking of TCR and MHC molecule on the peripheral B cells induced apoptosis. To assess MHC class II–mediated apoptosis, B cells were cocultured with two representative T cell clones, T23 (fails to provide MHC-restricted help) and T25 (provides MHC-restricted help). DNA content of B cells 40 h after initiation of the culture was determined by a two-color FACS® analysis, and the percentages of subdiploid, diploid, tetraploid B cells, and the B cells in the S phase were calculated (Fig. 3). Cultures including T cell clone T23 and superantigen contained 76% of subdiploid B cells, suggesting that these cells had undergone apoptosis. In contrast, only 28% of B cells were subdiploid after

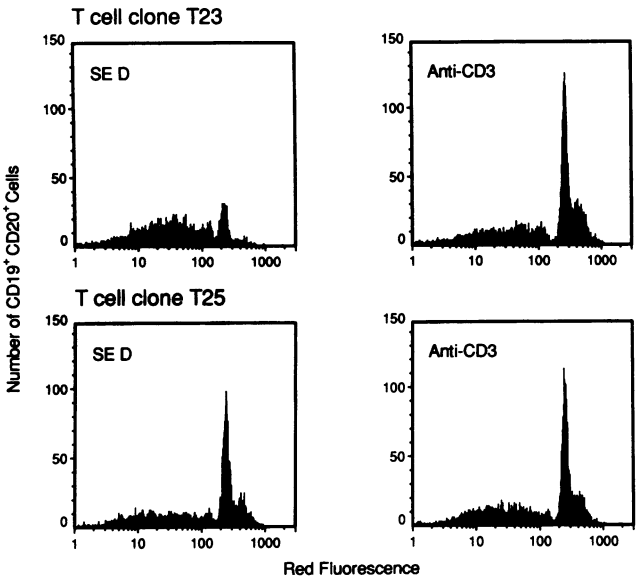

Figure 3. TCR-MHC cross-linking induces B cell apoptosis. T cell clones T23 (fails to provide MHC-restricted help) and T25 (provides MHC-restricted help) were cultured with nonstimulated peripheral B cells. T–B cell cultures were analyzed for B cell apoptosis after 40 h. Cells were stained with a mixture of anti-CD19-FITC, anti-CD20-FITC, and propidium iodide. The apoptotic B cells were identified by DNA content as determined by FACS® analysis. Data from one of five experiments using B cells from four different donors are shown. The T cell clones clearly differed in their ability to induce B cell apoptosis. T cell clone T23 induced apoptosis in the majority of B cells after stimulation with SE D, but not after stimulation with anti-CD3 (*top*). T cell clone T25 infrequently induced B cell apoptosis under both stimulation conditions.

stimulation with anti-CD3–activated T23 cells. Tetraploid B cells and B cells in the S phase indicating B cell differentiation and proliferation were encountered in anti-CD3–driven cultures (21%), but were essentially absent from SE D–driven cultures (3%). In contrast, the distributions of subdiploid, diploid, tetraploid B cells, and B cells in the S phase were very similar in T–B cell cultures involving T cell clone T25, independent of whether they were stimulated by superantigen or anti-CD3. A small fraction of B cells contained a subdiploid amount of DNA. The majority of B cells was either diploid or underwent proliferation.

To exclude the possibility that the purification of B cells with L-leucine methylester had rendered the B cells more susceptible to apoptosis, B cells were semipurified from PBMC by sequential removal of adhering cells and T cells and then cocultured with the T cell clones T23 and T25. These experiments confirmed the findings shown in Fig. 3. About 50% of all B cells underwent spontaneous apoptosis if cocultured with nonactivated T cells. Anti-CD3 stimulation of either T cell clone reduced the number of apoptotic B cells to 22 and 13%, respectively. The frequency of apoptotic B cells increased to 84% in the presence of SE D and T cell clone T23. In contrast, T cell clone T25 lacked the ability to induce B cell apoptosis. These data indicate that cross-linking of TCR and MHC class II molecules induces apoptosis of B cells. Only a subset of T cells can provide the apoptosis-inducing signal or, alternatively, a subset is not able to rescue B cells from the apoptosis-inducing signal.

Table II. IL-2, IFN-γ, and IL-4 Secretion of CD4⁺ T Cell Clones

Clone	Lymphokine secretion (U/ml)		
	IL-2	IFN-γ	IL-4
T02	nd*	8.21	4.48
T37	nd	1.24	3.03
T25	nd	0.10	2.29
T72	0.10	21.58	6.90
T32	nd	0.16	1.10
T08	32.26	450.00	3.47
T23	22.55	316.37	0.32
T15	63.56	74.23	1.70
T04	1.27	172.03	3.56
T38	132.24	188.32	8.07

* Not detected.

TH2, but not TH0, cells rescue B cells from MHC-mediated apoptosis. To identify features distinguishing the functionally different subset of T cell clones, lymphokine profiles of all T cell clones were determined. Results are given in Table II. The two T cell subsets could be differentiated based on their IL-2 and IFN-γ production. T cell clones which were able to provide help in SE D–driven cultures produced no, or very little, IL-2 and IFN-γ, while T cell clones that induced B cell apoptosis after TCR-MHC cross-linking produced high concentrations of IL-2 and IFN-γ. Both subsets of T cell clones were not different for the secretion of IL-4. The production of IL-4 ranged from 1.1 U/ml to 6.9 U/ml in the subset of T cell clones able to provide B cell help. T cell clones unable to promote MHC-restricted help secreted 0.32–8.07 U/ml IL-4. We explored the possibilities that different T cell stimuli could result in distinct lymphokine profiles. For all T cell clones tested, we found that the pattern of lymphokines produced were indistinguishable whether the T cells were activated with SE D or anti-CD3 (data not shown). In addition to the lymphokine secretion pattern shown in Table II, T cell clones were also analyzed for the expression of lymphokine mRNA transcripts by PCR (data not shown). The major difference in the two T cell subsets was the production of IL-2 mRNA and IFN-γ mRNA. There was no correlation with the expression of other lymphokines, including IL-4 and IL-10. All T cell clones expressed IL-4 and IL-10 transcripts upon activation.

To define the role of different lymphokines in the apoptosis of B cells, T cell clone T23 was cocultured with B cells and SE D in the presence of 2–10 U/ml exogenous IL-4 and supernatants from anti-CD3 activated TH2 T cell clones, respectively. Neither exogenous IL-4 nor the lymphokine mixture contained in the supernatant could restore the ability of the T cell clone to activate B cells and induce IgM production. Also, neither IL-2 in the concentrations of 10–100 U/ml, IFN-γ in the concentrations of 100–1,000 U/ml, TNF-α in the concentrations of 10–1,000 U/ml, nor 100% supernatant from activated TH0 cells was able to induce B cell apoptosis in cocultures including a TH2 T cell clone, B cells, and SE D (data not shown). These data suggest that the distinct lymphokine patterns observed for the two subsets of T cell clones were a hallmark distinguishing these two subsets. However, we do not have evidence that IL-

Table III. Induction of B Cell Apoptosis by Fixed Preactivated T Cells

T cell clone*	Functional phenotype	Percentage of apoptotic B cells	
		Without SE D	With SE D
T23	TH0	28	76
T23-fixed cells		33	48
T25	TH2	20	24
T25-fixed cells		34	30

* T cell clones were activated with immobilized anti-CD3.

2, IFN-γ, TNF-α, IL-4, or another soluble factor present in the supernatants was directly involved in the apoptotic events.

To directly address the issue that cell–cell interactions are important in the induction of apoptosis, the TH0 clone T23 and the TH2 clone T25 were activated with anti-CD3 and then fixed with formaldehyde. The fixed T cell clones were cocultured with B cells in the presence and absence of SE D, and the cultures were analyzed for B cell apoptosis after 36 h. For the TH2 T cell clone T25, the addition of SE D did not increase the number of apoptotic B cells (Table III). In contrast, the addition of SE D to cell cultures including fixed activated cells of the T cell clone T23 increased the number of apoptotic B cells from 33 to 48%. The increase in apoptotic B cells, although less pronounced than the effect of viable T23 cells, was a consistent finding in two experiments (data not shown). These results support the interpretation that differences in the membrane structure of TH0 and TH2 clones are related to the ability to induce apoptosis in B cells.

Activated B cells are not susceptible to apoptosis. It has been shown that activated B cells are refractory to MHC class II–mediated apoptosis (14). Also, cross-linking of cell surface immunoglobulin can protect B cells from apoptosis (20). We wanted to address the question whether only peripheral B cells, which had not been activated in vitro, were susceptible to the apoptosis-inducing signals delivered by SE D and TH0-like T cell clones. T cell clone T23 was activated with immobilized anti-CD3 and cocultured with freshly purified peripheral B cells. 0.5 μg/ml SE D was added at different time points as indicated in Fig. 4, and supernatants were assayed for immunoglobulin production after a total culture period of 14 d. Production of significant concentrations of immunoglobulins in these T-B cell cultures requires a minimum culture period of 10 d. Maximum immunoglobulin levels are reached by day 14 (21). The T cell clone that failed to induce IgM production in B cells in the presence of SE D was a potent helper cell for nonspecific contact help elicited by anti-CD3 activation. Addition of SE D to anti-CD3–driven cultures showed that SE D completely inhibited IgM production when added as late as day 2 and partially inhibited the induction of IgM when added at day 4. No inhibitory effect was seen after day 4. These data demonstrate that B cells are susceptible to apoptosis in the first 2–4 d after coculturing with anti-CD3–activated T helper cells. These data also indicate that T helper cells, which are fully activated two days after anti-CD3 stimulation, are still able to induce B cell apoptosis and do not rescue B cells from the apoptosis inducing mechanism.

Discussion

The capacity of B cells to process and present antigen to T helper cells has been understood as a fundamental mechanism

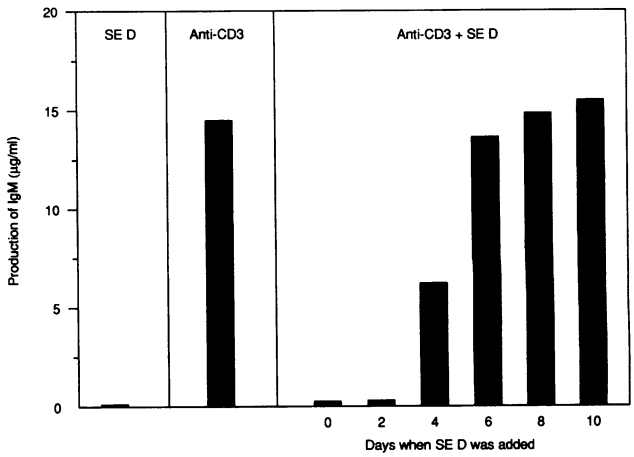

Figure 4. Activated B cells are not susceptible to MHC-mediated apoptosis. B cells cocultured with T cell clone T23 were stimulated by either SE D (*left*), anti-CD3 (*middle*), or anti-CD3 and SE D. SE D was added at different time points after initiation of the culture as indicated (*right*). SE D completely inhibited IgM production when added as late as day 2. No inhibitory effect was found when SE D was added after day 4.

ensuring targeting of helper signals to the appropriate B cell (22, 23). Recently, however, MHC class II molecules have not only been implicated in transmitting positive signals to the B cell, but ligation of MHC class II molecules by mAb has been found to mediate apoptotic death of B cells (14). Here, we demonstrate that TCR-MHC–mediated interaction between T helper cells and peripheral human B cells can result in B cell apoptosis or B cell differentiation/proliferation, depending on the functional commitment of the T cell providing B cell help. We were able to link the induction of B cell apoptosis with the lymphokine patterns of the interacting T helper cells. B cells interacting with a TH0 helper cell undergo apoptosis, while TH2 helper cells induce immunoglobulin synthesis after superantigen-mediated interaction. Both types of helper cells support B cell differentiation and growth in an MHC-independent, antigen-nonspecific way as facilitated by anti-CD3 stimulation of the T cells. B cell apoptosis induced by TCR-MHC cross-linking cannot be influenced by exogenous T cell–derived lymphokines, but appears to be the consequence of cell–cell contact. In vitro activated B cells are protected from apoptotic cell death and are able to receive help from functionally distinct helper cells.

Two different models could explain why B cells undergo apoptosis in the presence of some, but not all, T helper cells. B cells have been shown to spontaneously undergo apoptosis in in vitro culture and signals from the T cell may be necessary to rescue the B cells from programmed cell death (24). It could be argued that B cells insufficiently activate TH0 cells and thus cannot be rescued from programmed cell death. Resting B cells are indeed poor antigen-presenting cells for primary T cell responses. Jenkins and Schwartz have provided evidence that stimulation of the TCR of TH1 T cells, in the absence of costimulatory signals, results in the induction of anergy in the responding T cell population (25–27). Anergic T cells are characterized by the lack of production of IL-2, but they do produce IFN-γ and express IL-2 receptors. Thus, T–B cell interaction may result in a very different outcome depending on the lym-

phokine profile of the T cell. TH0 and TH2 cells may have different stimulation requirements and the stimulation by resting B cells and superantigen may not be sufficient to activate TH0 cells to be efficient helper cells in T–B cell interaction. Alternatively, the apoptotic B cell death could be the result of signals provided by the interacting T cell. To distinguish between these two models, we combined the two different stimuli which either lead to B cell depletion (superantigenic stimulation) or B cell differentiation (stimulation with immobilized anti-CD3). The addition of superantigen to anti-CD3–stimulated cultures suppressed the induction of immunoglobulin secretion in the presence of TH0 cells, but not TH2 cells. These data clearly showed that the superantigen-mediated effect is an active and dominant phenomenon, suggesting that the cross-linking of the TCR on the TH0 cell, and the HLA-DR molecule on the B cell, induces B cell apoptosis.

The engagement of the TCR with the HLA molecule either results in B cell apoptosis or B cell stimulation. The functional commitment of the interacting T cell appears to determine the outcome by providing an active signal. It remains possible that both TH0 and TH2 cells are able to induce MHC-mediated apoptosis, however, only TH2 cells deliver the additional signals necessary to rescue the B cells from apoptosis. A critical role of T cell–derived cytokines in B cell apoptosis could be postulated. Thus, we have tested the question whether TH0- or TH2-derived lymphokines were involved in the induction of or protection from apoptosis. All T cell clones, including TH0 and TH2 cells, produced IL-10, suggesting that the production of IL-10 did not account for the lack of B cell apoptosis observed with TH2 cells. Several groups have shown that IL-4, at least in combination with antiimmunoglobulin or with anti-CD40 can prevent B cell apoptosis (7, 14, 24). However, the production of IL-4 was rather similar in both subsets of T cells, suggesting that IL-4 did not play a role in the prevention of apoptosis. In support of this view, the addition of exogenous IL-4 or supernatants from activated TH2 cells could not induce differentiation of B cells cocultured with TH0 cells, suggesting as one possibility that soluble factors derived from TH2 cells were not sufficient to prevent apoptosis. We also explored the possibility that IFN-γ was directly involved in apoptosis. However, exogenous IFN-γ was insufficient to prevent a productive interaction between TH2 cells and B cells, and to induce B cell apoptosis. Also, TNF-α, which has been shown to induce apoptosis (28), did not increase the number of apoptotic B cells when added to cultures consisting of TH2 cells, B cells, and SE D.

Our experiments strongly suggest that the interaction between TCR and MHC molecules is directly involved in inducing B cell apoptosis. However, we cannot exclude that TH0 and TH2 cells differ in the expression or induction of cell surface molecules which are important to mediate apoptosis. As one possibility, the stimulation of the APO-1 or Fas antigen has been shown to induce cell death by apoptosis in some cell types (29, 30). To address the question of whether TH0 cells and TH2 cells differ in the expression of the Fas ligand, we tested the capacity of TH0 and TH2 cells to induce apoptosis of P815 cells transfected with the human Fas antigen (kindly provided by C. Eischen and P. Leibson, Department of Immunology, Mayo Clinic, Rochester, MN). These experiments did not demonstrate a difference between TH0 and TH2 cells, suggesting that the discriminating factor between TH0 and TH2 cells is not the expression of the Fas ligand.

Differences in the outcome of T–B cell interaction resulting in either immunoglobulin production or B cell depletion have been described (31). Del Prete et al. have demonstrated that most TH1 cells and some TH2 cells exhibited cytolytic activity for B cells. These authors report that all TH1 T cell clones lysed Epstein-Barr virus transformed autologous B cells pulsed with the specific antigen. We tested the question whether the induction of apoptosis was associated with the ability of TH0 cells to lyse B cells. However, we were unable to demonstrate that any of the $CD4^+$ T cell clones examined had a lytic machinery. Neither the presence of lectin nor the presence of superantigen was able to facilitate cell lysis of target cells in chromium release assays (data not shown). We tested several different types of target cells, including resting B cells, pokeweed mitogen–activated B cell blasts, Epstein-Barr virus–transformed B cells, and mouse tumor lines with established sensitivity to T cell–mediated killing. In line with our finding that the induction of apoptosis was not due to the cytolytic mechanism described by Del Prete et al., we found that preactivated B cells were protected from cell death. Also, kinetic studies exploring the number of surviving B cells in the cultures, as well as monitoring B cell apoptosis, revealed that B cell death was a delayed phenomenon which could not be detected in the 6-h cytolytic assay. Culture periods of 24 h were insufficient to demonstrate the decline in B cell numbers. In summary, the B cell apoptosis we have observed upon MHC-mediated interaction with TH0 cells appears to be distinct from the cell lysis mediated by TH1 cells as reported by Del Prete et al (31).

Induction of B cell apoptosis as a result of T cell recognition raises the question of how this mechanism contributes to the regulation of antibody production. Engagement of the TCR with the HLA complex provides specificity control in T–B cell interaction and ascertains that appropriate help is directed to a B cell–presenting antigen. Apoptosis has recently attracted attention as a mechanism, deleting unwanted cells in the immune system (32, 33). Antigen-specific interaction of resting B cells with T cells has been described as a means to induce T cell tolerance, suggesting as one model, that recognition of antigens presented by such B cells carries the risk of autoreactivity (34, 35). Induction of T cell anergy has been associated with antigen recognition in the absence of appropriate costimulatory signals (25–27, 36). While interaction of a resting B cell with a T cell may prevent T cell activation by the induction of anergy, such a contact could be sufficient to induce the expression of costimulatory molecules in the B cell. Studies by Nabavi et al. and by Watts et al. have demonstrated that expression of the B cell accessory molecule B7 is regulated through contact of the TCR with the MHC antigen ligand (37, 38). Subsequent T–B cell interactions of such a B cell could then fail to induce anergy. We are proposing that apoptotic cell death of B cells provides a mechanism to prevent the aberrant induction of costimulatory molecules on B cells and the subsequent activation of potentially autoreactive T cells.

Data presented here are consistent with the notion that the strict control of T–B cell interaction only applies to the initial stages of the immune response (35, 39, 40). As soon as B cells are activated, they cannot only provide costimulatory signals to T cells to promote T cell activation, but they are also no longer susceptible to the apoptosis inducing T cell–derived signals. Also, B cell apoptosis as a tolerance mechanism is no longer effective as soon as T cells have differentiated into TH2 cells. TH2 cells promote differentiation of nonstimulated, as well as

activated, B cells in the superantigen-mediated T–B cell interaction.

Many autoimmune diseases are characterized by the abundant production of antibodies including autoantibodies. Very little information is available on mechanisms involved in breaking tolerance. Our model would suggest that recognition of autoantigens on resting B cells is well controlled: antigen-presenting resting B cells are depleted from the pool after interaction with TH0 cells and the interacting TH0 cells are rendered anergic and do not differentiate into TH2 cells. Failure in either mechanism could result in uncontrolled activation of autoreactive B cells and T cells.

Acknowledgments

The authors wish to thank Toni L. Higgins for secretarial assistance.

This work was supported by Minnesota Arthritis Foundation grant No. 84, Lupus grant No. 4, and the Mayo Foundation.

References

1. Kehry, M. R., and P. D. Hodgkin. 1993. Helper T cells: delivery of cell contact and lymphokine-dependent signals to B cells. *Semin. Immunol.* 5:393–400.

2. Parker, D. C. 1993. T cell-dependent B cell activation. *Annu. Rev. Immunol.* 11:331–360.

3. Armitage, R. J., C. R. Maliszewski, M. R. Alderson, K. H. Grabstein, M. K. Spriggs, and W. C. Fanslow. 1993. CD40L: a multi-functional ligand. *Semin. Immunol.* 5:401–412.

4. Hallenbaugh, D., L. S. Grosmaire, C. D. Kullas, N. J. Chalupny, S. Braesch-Andersen, R. J. Noelle, I. Stamenkovic, J. A. Ledbetter, and A. Aruffo. 1992. The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell costimulatory activity. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:4313–4321.

5. Clark, E. A., and J. A. Ledbetter. 1986. Activation of human B cells mediated through two distinct cell surface differentiation antigens, Bp35 and Bp50. *Proc. Natl. Acad. Sci. USA* 83:4494–4498.

6. Stamenkovic, I., E. A. Clark, and B. Seed. 1989. A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1403–1410.

7. Banchereau, J., P. de Paoli, A. Valle, E. Garcia, and F. Rousset. 1991. Long term human B cell lines dependent on interleukin-4 and antibody to CD40. *Science (Wash. DC).* 251:70–72.

8. Rousset, F., E. Garcia, and J. Banchereau. 1991. Cytokine-induced proliferation and immunoglobulin production of human B lymphocytes triggered through their CD40 antigen. *J. Exp. Med.* 173:705–710.

9. Noelle, R. J., M. Roy, D. M. Shepherd, I. Stamenkovic, J. A. Ledbetter, and A. Aruffo. 1991. A 39-kDa protein on activated helper T cells binds CD40 and transduces the signal for cognate activation of B cells. *Proc. Natl. Acad. Sci. USA* 89:6550–6554.

10. Armitage, R. J., W. C. Fanslow, L. Strockbine, T. A. Sato, K. N. Clifford, B. M. Macduff, D. M. Anderson, S. D. Gimpel, T. Davis-Smith, C. R. Maliszewski, et al. 1992. Molecular and biological characterization of a murine ligand for CD40. *Nature (Lond.).* 357:80–82.

11. Grabstein, K. H., C. R. Maliszewski, K. Shanebeck, T. A. Sato, M. K. Spriggs, W. C. Fanslow, and R. J. Armitage. 1993. The regulation of T cell dependent antibody formation in vitro by CD40 ligand and interleukin 2. *J. Immunol.* 150:3141–3147.

12. Parker, D. C. 1993. The functions of antigen recognition in T cell-dependent B cell activation. *Semin. Immunol.* 5:413–420.

13. Forsgren, S., G. Pobor, A. Coutinho, and M. Pierres. 1984. The role of I-A/E molecules in B lymphocyte activation. I. Inhibition of lipopolysaccharide-induced responses by monoclonal antibodies. *J. Immunol.* 133:2104–2110.

14. Newell, M. K., J. VanderWall, K. S. Beard, and J. H. Freed. 1993. Ligation of major histocompatibility complex class II molecules mediates apoptotic cell death in resting B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 90:10459–10463.

15. He, X., J. J. Goronzy, and C. M. Weyand. 1992. Selective induction of rheumatoid factors by superantigens and human helper T cells. *J. Clin. Invest.* 89:673–680.

16. Thiele, D. L., and P. E. Lipsky. 1986. The immunosuppressive activity of L-leucyl-L-leucine methylester: selective ablation of cytotoxic lymphocytes and monocytes. *J. Immunol.* 136:1038–1048.

17. Dent, G. A., M. C. Leglise, K. B. Pryzwansky, and D. W. Ross. 1989. Simultaneous paired analysis by flow cytometry of surface markers, cytoplasmic antigens, or oncogene expression with DNA content. *Cytometry.* 10:192–198.

18. Custer, M. C., and M. T. Lotze. 1990. A biologic assay for IL-4. Rapid fluorescence assay for IL-4 detection in supernatants and serum. *J. Immunol. Methods.* 128:109–117.

19. Gold, K. N., C. M. Weyand, and J. J. Goronzy. 1994. Modulation of helper T cell function by prostaglandins. *Arthritis Rheum.* 37:925–933.

20. Kobayashi, R., L. Z. Rassenti, G. Meisenholder, D. A. Carson, and T. J. Kipps. 1993. Autoantigen inhibits apoptosis of a human B cell leukemia that produces pathogenic rheumatoid factor. *J. Immunol.* 151:7273–7283.

21. He, X., J. J. Goronzy, and C. M. Weyand. 1993. The repertoire of rheumatoid factor producing B cells responsive to bacterial superantigen and bystander help in normals and patients with rheumatoid arthritis. *Arthritis Rheum.* 36:1061–1069.

22. Chesnut, R. W., and H. M. Grey. 1981. Studies on the capacity of B cells to serve as antigen-presenting cells. *J. Immunol.* 126:1075–1079.

23. Lanzavecchia, A. 1985. Antigen-specific interaction between T and B cells. *Nature (Lond.).* 314:537–539.

24. Illera, V. A., C. E. Perandones, L. L. Stunz, D. A. Mower, Jr., and R. F. Ashman. 1993. Apoptosis in splenic B lymphocytes. Regulation by protein kinase C and IL-4. *J. Immunol.* 151:2965–2973.

25. Jenkins, M. K., J. D. Ashwell, and R. H. Schwartz. 1988. Allogeneic non-T spleen cells restore the responsiveness of normal T cell clones stimulated with antigen and chemically modified antigen-presenting cells. *J. Immunol.* 140:3324–3330.

26. Schwartz, R. H. 1990. A cell culture model for T lymphocyte clonal anergy. *Science (Wash. DC).* 248:1349–1355.

27. Schwartz, R. H. 1992. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell.* 71:1065–1068.

28. Hernandez-Caselles, T., and O. Stutman. 1993. Immune functions of tumor necrosis factor. I. Tumor necrosis factor induces apoptosis of mouse thymocytes and can also stimulate or inhibit IL-6-induced proliferation depending on the concentration of mitogenic costimulation. *J. Immunol.* 151:3999–4012.

29. Yonehara, S., A. Ishii, and M. Yonehara. 1989. A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor. *J. Exp. Med.* 169:1747–1756.

30. Trauth, B. D., C. Klas, A. M. Peters, S. Matzku, P. Moller, W. Falk, K. M. Debatin, and P. H. Krammer. 1989. Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science (Wash. DC).* 245:301–305.

31. Del Prete, G. F., M. De Carli, M. Ricci, and S. Romagnani. 1991. Helper activity for immunoglobulin synthesis of T helper type 1 (Th1) and TH2 human T cell clones: the help of Th1 clones is limited by their cytolytic capacity. *J. Exp. Med.* 174:809–813.

32. Cohen, J. J., R. C. Duke, V. A. Fadok, and K. S. Sellins. 1992. Apoptosis and programmed cell death in immunity. *Annu. Rev. Immunol.* 10:267–293.

33. Green, D. R., and T. G. Cotter. 1992. Introduction: apoptosis in the immune system. *Semin. Immunol.* 4:355–362.

34. Eynon, E. E., and D. C. Parker. 1992. Small B cells as antigen-presenting cells in the induction of tolerance to soluble protein antigens. *J. Exp. Med.* 175:131–138.

35. Fuchs, E. J., and P. Matzinger. 1992. B cells turn off virgin but not memory T cells. *Science (Wash. DC).* 258:1156–1159.

36. Liu, Y., and P. S. Linsley. 1992. Costimulation of T-cell growth. *Curr. Opin. Immunol.* 4:265–270.

37. Nabavi, N., G. J. Freeman, A. Gault, D. Godfrey, L. M. Nadler, and L. H. Glimcher. 1992. Signaling through the MHC class II cytoplasmic domain is required for antigen presentation and induces B7 expression. *Nature (Lond.).* 360:266–268.

38. Watts, T. H., N. Alaverdi, W. F. Wade, and P. S. Linsley. 1993. Induction of costimulatory molecule B7 in M12 B lymphomas by cAMP or MHC-restricted T cell interaction. *J. Immunol.* 150:2192–2202.

39. Tite, J. P., J. Kaye, and B. Jones. 1984. The role of B cell surface Ia antigen recognition by T cells in B cell triggering: analysis of the interaction of cloned helper T cells with normal B cells in differing states of activation and with B cells expressing the xid defect. *Eur. J. Immunol.* 14:553–561.

40. Ronchese, F., and B. Hausmann. 1993. B lymphocytes in vivo fail to prime naive T cells but can stimulate antigen-experienced T lymphocytes. *J. Exp. Med.* 177:679–690.