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LYMPHOKINE CONTROL OF TYPE 2 ANTIGEN RESPONSE

IL-10 Inhibits IL-5- but not IL-2-Induced Ig Secretion by T Cell-Independent Antigens[1]

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A supernatant derived from the Th2 clone D10.G4.1 (D10 supernatant) stimulated high numbers of Ig-secreting cells when added to dextran-conjugated anti-δ-antibody (anti-δ-dextran)-activated B cells but stimulated only marginal Ag-specific responses when added to B cells cultured with TNP-Ficoll. When anti-IL-10 antibody was added to cultures containing D10 supernatant, IL-5, and TNP-Ficoll, there was a significant increase in the numbers of anti-TNP-antibody producing cells, suggesting that at least a part of the inhibitory activity of D10 supernatant is mediated by IL-10. Addition of rIL-10 inhibited both TNP-Ficoll- and anti-δ-dextran-mediated Ig secretion that was stimulated in the presence of IL-5 but had no suppressive effect on IL-2-stimulated responses, indicating that its inhibitory effect was selective for a specific mode of B cell activation. Addition of IL-10 did not, however, inhibit anti-δ-dextran-stimulated B cell proliferation. The IL-10-induced-inhibition of Ig secretion was not due to suppression of IFN-γ production, because the addition of IFN-γ did not reverse the inhibition, nor did the addition of anti-IFN-γ mimic the IL-10-mediated inhibition. These data suggest that a composite of lymphokines secreted by Th cells may contain both inhibitory and stimulatory activities. Sorting out the conditions under which stimulation or inhibition is seen may reveal additional diversity in Ag-stimulated pathways of B cell activation.

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We have previously shown that TNP-Ficoll, a prototypical TI-2[4] Ag, is unable to stimulate in vitro anti-TNP responses by resting B cells unless lymphokines are added (1, 2). Thus, although this class of polysaccharide Ag is unable to stimulate Ag-specific T cells, it is absolutely dependent on a source of cytokines to stimulate Ig secretion. Both IL-5 (3, 4) and IL-2 (2, 4, 5) have recently been shown to synergize with this class of Ag to stimulate Ig secretion. Because analysis of B cell responses to TI-2 Ag has been hampered by the low frequency of Ag-specific B cells found in unimmunized mice, we designed a model system to study, at a polyclonal level, B cell activation that was stimulated by agonists that resembled TI-2 Ag. We demonstrated that B cells stimulated by anti-δ-dextran responded, in many respects, like B cells that were activated by TNP-Ficoll (6). Thus, like TNP-Ficoll, anti-δ-dextran stimulated resting B cells to secrete Ig only in the presence of IL-2 or IL-5; similarly, both TNP-Ficoll and anti-δ-dextran induced Ig secretion by large preactivated B cells in the absence of added cytokines (6, 7). These studies demonstrated that lymphokines derived from either $CD4^+$ Th1 or Th2 T cells may play a significant role in the humoral response of resting B cells to TI-2 Ag.

In the course of studies designed to evaluate the effects of the Th1- and Th2-derived lymphokines on TI-2-induced responses in vitro, we found that, although rIL-5 enhanced TNP-Ficoll-stimulated responses in vitro, supernatants derived from a Th2 line that also contained high concentrations of IL-5 were strongly inhibitory. In this report, we demonstrate that this inhibitory activity in Th2 cell supernatant reflects, in part, the presence of IL-10.

MATERIALS AND METHODS

Mice. Female DBA/2 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were used at 6 to 8 weeks of age.

Antibodies and other reagents. The anti-T cell mAb anti-Thy-1.2 (clone 30-H12) (8), anti-CD4 (clone GK1.5) (9), and anti-CD8 (clone 53-6.7) (9) were grown as ascitic fluid in nude mice and purified as described previously (10). The mouse mAb against rat IgG κ-chain, MAR 18.5 (11), was produced by cells grown in tissue culture. Mouse anti-IgD mAb $H\delta^a/1$ (12) was purified from ascitic fluid as described (10). $H\delta^a/1$ was conjugated to a high m.w. dextran (2×10^6 Da), at a ratio of $H\delta^a/1$ to dextran of 6:1 (13). Concentrations of dextran-conjugated antibodies that are noted in the text reflect only the anti-Ig antibody concentration and not that of the entire dextran conjugate.

[4] Abbreviations used in this paper: TI-2, T-independent type 2; anti-δ-dextran, dextran-conjugated anti-Ig antibody; D10, D10.G4.1 T cell clone; PFC, antibody-producing cells.

Purified SCX1 (rat IgM anti-IL-10) (14) was obtained by ammonium sulfate precipitation of supernatants from hybridoma cells grown in serum-free medium. The rat IgM antibody J5 (14), obtained in a similar way, was used as a control. Both antibodies were kindly provided by Dr. Alan Sher (National Institutes of Allergy and Infectious Diseases, National Institutes of Health). Purified XMG-6 (rat IgG1 anti-murine IFN-γ) (15) and the control antibody J4-1 (rat IgG1 anti-NP) (16) were purified from ascites as described (10). Both antibodies were kindly provided by Dr. Fred Finkelman (Uniformed Services University, Bethesda, MD). rIL-5 was produced by using the recombinant baculovirus AeNPV-IL-5 in an SF9 cell system and was purified by using TRFK-5 (rat IgG1 anti-IL-5 mAb)-Sepharose, as described (17). The rIL-5 was a kind gift of Dr. G. Harriman (National Institutes of Allergy and Infectious Diseases, National Institutes of Health). One unit of activity was equal to 125 pg of rIL-5. Units were determined in the BCL_1 assay (17). rIL-10 was obtained by transfecting COS-7 cells with the F115 cDNA clone, as previously described (18). Supernatant from this transfection was used in all experiments. Supernatants obtained from mock transfections were used as control. These IL-10-containing supernatants were kindly provided by Dr. Alan Sher and Dr. Maureen Howard (DNAX, Palo Alto, CA). TNP-Ficoll was prepared as previously described (19).

B cell purification. Suspensions of single spleen cells were washed three times with RPMI 1640 (M. A. Bioproducts. Walkersville, MD) plus 10% FCS (GIBCO, Grant Island, NY) and were treated (10^7 spleen cells/ml) with anti-Thy-1.2 (1 μg/ml), anti-CD4 (2.5 μg/ml), and anti-CD8 (1/600 dilution of ascitic fluid) mAb, for 30 min on ice. This was followed by treatment with newborn rabbit C (10%) (Pel-Freez, Rogers, AR), in the presence of a 1/10 dilution of tissue culture fluid containing the anti-rat κ-chain mAb MAR 18.5, at 37°C for 45 min.

Cells were then fractionated into low and high density populations by Percoll (Pharmacia, Uppsala, Sweden) gradient centrifugation (3000 rpm for 15 min). Gradients consisting of 70, 65, 60, and 50% Percoll (with densities of 1.086, 1.081, 1.074, and 1.062 g/ml, respectively) were used. The high density cells (resting) were collected from the 70 to 65% interface, and the low density cells were collected from the 50 to 60% interface. The average percentage of Ig^+ cells in the 70 to 65% fractions was 90 to 95%.

B cell sorting. High density, resting B cells were washed two times in cold HBSS (without phenol red) plus 3% FCS. Cells were stained with phycoerythrin-labeled polyclonal goat anti-mouse IgM (Southern Biotechnology Associates, Birmingham, AL). IgM^+ cells were isolated by electronic cell sorting, by using logarithmic amplification, on a FACStar Plus (Becton-Dickinson, Mountain View, CA). Residual dead cells and macrophages were eliminated on the basis of their characteristic forward and side scatter profiles. Sorted cells were immediately reanalyzed to confirm their staining profile and were found to be >99% IgM^+. Sorted cells were cultured immediately after their isolation.

Measurement of antibody-producing cells. B cells were cultured for 4 to 6 days, in a final volume of 0.2 ml of modified Mishel-Dutton medium, in flat-bottomed 96-well trays (Costar, Cambridge, MA). B cells secreting anti-TNP antibodies were enumerated with a hemolytic plaque assay, by using TNP-conjugated SRBC, as described (20). Antibody-producing cells were assayed by a protein-A-SRBC plaque assay (21). IgM levels in these cultures were determined by ELISA, as previously described (22).

Preparation of $CD4^+$ T cell supernatant. Cells from the conalbumin-specific $CD4^+$ T cell clone D10 (23) were cultured, at 10^5/ml, for 24 h with conalbumin (100 μg/ml) and 5×10^5 T-depleted, 3000-rad-irradiated, spleen cells from C3H mice, as a source of APC. The cell-free supernatant was kept frozen at −80°C until used. D10 is defined as a Th2 clone, based on its secretion of IL-4, IL-5, and IL-6 and the absence of IL-2 and IFN-γ production (15). The batches of D10 supernatant used in this report had, on average, 95 U/ml IL-5 and 365 U/ml IL-4. The concentrations of both lymphokines were determined by ELISA (24), with reagents kindly provided by Dr. G. Harriman (National Institutes of Allergy and Infectious Diseases, National Institutes of Health).

Measurement of [^{3}H]Tdr incorporation. B cells were cultured for 48 h, in a final volume of 0.2 ml of modified Mishel-Dutton medium, in flat-bottomed 96-well trays (Costar). [^{3}H]Tdr (1.0 μCi) (Amersham, Arlington Heights, IL), with a specific activity of 20 Ci/mmol, was added to the cultures for the final 18 h, and cultures were harvested onto glass fiber filters, by using a PHD cell harvester (Cambridge Technology, Watertown, MA). Specific incorporation of Tdr was analyzed by liquid scintillation counting, and results are expressed as the arithmetic mean of triplicate cultures.

RESULTS

Inhibition of the lymphokine-stimulated anti-TNP-Ficoll response by a Th2 supernatant. Supernatant from the Th2 clone D10 induced high levels of Ig secretion by cells stimulated with anti-δ-dextran (Table I) (6). Because we have previously described this conjugate as a useful model for studying responses to TI-2 Ag; it was important to test whether this source of lymphokines could also induce Ig secretion by TNP-Ficoll-stimulated B cells. B cells were cultured with TNP-Ficoll in the presence of IL-5 or D10 supernatant. Although IL-5 stimulated anti-TNP antibody secretion in vitro (Table II, Experiment I), D10 supernatant containing similar concentrations of IL-5 was poorly stimulatory and, in fact, inhibited the response that was induced by TNP-Ficoll and IL-5. Although the IL-5-stimulated TNP-Ficoll response was inhibited by D10 supernatant, the IL-2-stimulated response was enhanced by this Th2-derived supernatant (Table II). The D10-mediated inhibition was not reversed even when higher concentrations of IL-5 (500 U/ml) were used (Fig. 1). Experiments with supernatants from another Th2 cell clone (CDC35) (25) showed similar results (data not shown).

Although our T-depleted B cell cultures contained 90 to 95% Ig^+ cells, it was possible that the remaining 5 to 10% contaminating non-B-non-T cells might be required for D10 supernatant-mediated inhibition to occur. To study this possibility, highly purified B cells were obtained by sorting IgM^+ cells on a FACS. D10 supernatant supported, to a very limited degree, TNP-Ficoll-stimulated antibody production in this population of 99% Ig^+ cells and still mediated marked inhibition of the IL-5-stimulated TNP-Ficoll response (Fig. 2). This demonstrates that

TABLE I

Response of B cells to anti-δ-dextran in the presence of D10 supernatant[a]

Stimuli	Ig-Producing Cells/Culture
Medium	18
D10 supernatant	74
Anti-δ-dextran + D10 supernatant	8,106

[a] Resting B cells (2.5×10^4) were cultured in 0.2 ml with anti-δ-dextran, in the presence of D10 supernatant. Anti-δ-dextran was used at 10 ng/ml and D10 supernatant at 20% final concentration. Antibody-producing cells were measured 5 days after initiation of culture.

TABLE II

Inhibition of the anti-TNF-Ficoll response by D10 supernatant and reversal of the inhibition by anti-IL-10 antibody[a]

Stimuli	Anti-TNP PFC/Culture	
	Medium	D10 Supernatant
Experiment I		
TNP-Ficoll	3 ± 1	74 ± 2
TNP-Ficoll + IL-5	380 ± 20	97 ± 19
TNP-Ficoll + IL-2	182 ± 18	872 ± 40
Medium	4 ± 3	55 ± 3
Experiment II		
TNP-Ficoll + IL-5 + control antibody	720 ± 5	72 ± 2
TNP-Ficoll + IL-5 + anti-IL-10	816 ± 64	552 ± 40
TNP-Ficoll	8 ± 4	60 ± 19
Medium	2 ± 4	18 ± 7

[a] Resting B cells (10^6) were stimulated with the indicated stimuli for 4 days. TNP-Ficoll was used at 20 ng/ml (Experiment I) or at 10 ng/ml (Experiment II). IL-5 was used at 20 U/ml and induced 33 PFC in Experiment I and 68 PFC in Experiment II. IL-2 was used at 100 U/ml and induced 23 PFC. Anti-IL-10 (SCX1) and control (J5) antibodies were used at 20 μg/ml and induced 4 and 8 PFC, respectively. Values are mean ± SD.

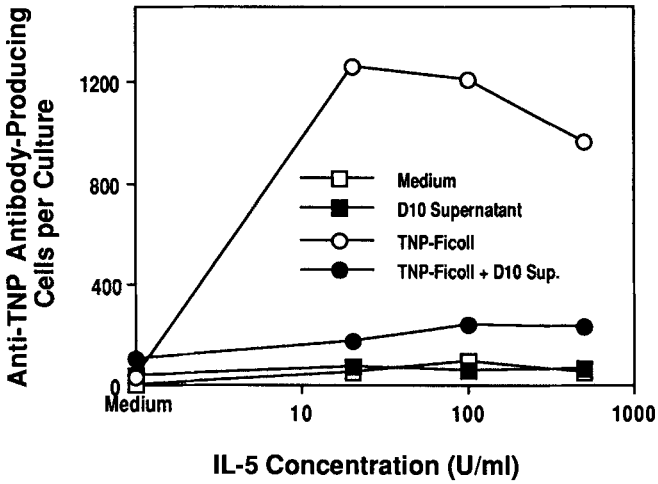

Figure 1. Inhibition of the anti-TNP-Ficoll response by D10 supernatant in the presence of different IL-5 doses. B cells (10^6) were stimulated with TNP-Ficoll (10 ng/ml), in the absence or presence of D10 supernatant (20%), for 4 days. In some experiments IL-5, at the indicated concentrations, was added. The values represent the mean of triplicate cultures, and the SD (not shown) was <10% for all points shown.

the inhibitory effect of D10 supernatant is mediated at the level of the B cell.

IL-10 is the inhibitory mediator present in D10 supernatant. To characterize the factors responsible for the D10-mediated inhibition of Ig production, we attempted to reverse this inhibition by adding antibodies to neutralize specific lymphokines known to be present in D10 supernatant. Addition of either anti-IL-4 or anti-transforming growth factor-β mAb was unable to reverse the D10 supernatant-induced inhibition (data not shown). However, addition of anti-IL-10 antibody in three of three experiments reversed the D10-mediated inhibition of the TNP-Ficoll- plus IL-5-induced antibody response (Table II, Experiment II).

To explore this further, we tested whether IL-10 alone could inhibit the TNP-Ficoll- plus IL-5-mediated antibody response. IL-10, at concentrations ranging from 5 to 50 U/ml, inhibited the IL-5-induced TNP-Ficoll response in a dose-dependent manner (Fig. 3*A*). Although IL-10 induced profound inhibition in the response induced by TNP-Ficoll and IL-5, it had no effect on the response induced by TNP-Ficoll and IL-2 (Fig. 3*B*). This IL-10-induced inhibition was specific, because it was reversed when anti-IL-10 mAb, but not control antibody, was added to the cultures (data not shown). Similar results were observed in the Ig-secretory responses stimulated by anti-δ-dextran; IL-10 inhibited IL-5- but not IL-2-enhanced Ig secretion (Table III). Experiments with mock supernatant in control cultures did not modify the response induced by anti-δ-dextran in the presence of IL-5 or IL-2.

Characterization of the mechanism of IL-10-mediated inhibition of Ig secretion. The finding that IL-10 was selective in its suppression of IL-5- and not IL-2-mediated responses suggested that it was probably not suppressing anti-Ig-mediated B cell proliferation. The data in Table IV demonstrate that IL-10 had little, if any, suppressive effect on anti-δ-dextran-stimulated B cell proliferative responses, even in the presence of IL-5. Although these early steps of B cell activation were not inhibited by IL-10, maximum inhibition of Ig secretion was, nonetheless, observed when IL-10 was added at the onset of culture, immediately before the addition of anti-δ-dextran (Fig. 4). Although delayed addition of IL-10 at day 4 of a 6-day culture reduced the degree of suppression, there was still a >60% suppression of Ig secretion, compared with control responses (Fig. 4).

IL-10 was previously shown to inhibit macrophage but not B cell Ag presentation to T cells (26). Although responses we were studying were macrophage independent, we wished to exclude a role for these cells in IL-10-mediated inhibition. When low density cells (which contained small numbers of macrophages) were added to cultures stimulated with TNP-Ficoll in the presence of IL-10, there was no demonstrable increase in the inhibitory effect of the anti-TNP responses (data not shown).

The finding that IFN-γ is produced during responses stimulated by TI-2 Ag (27) and that it may have stimulatory effects on responses to these Ag (2) suggested the possibility that IL-10-mediated inhibition reflected its ability to inhibit IFN-γ secretion by non-B cells. Although we regard this as unlikely, because sorted IgM^+ cells were inhibited by D10 supernatant (Fig. 2), we, nevertheless, studied whether addition of IFN-γ would overcome the inhibitory effect of IL-10 or whether anti-IFN-γ would mimic the inhibitory effect seen with IL-10 (Table V). Addition of anti-IFN-γ or rIFN-γ did not modify the pattern of response induced by either TNP-Ficoll or anti-δ-dextran. This indicates that IL-10-induced inhibition is not mediated by suppression of IFN-γ production.

DISCUSSION

We demonstrate that Th2-derived supernatant, although promoting Ig secretion by anti-δ-dextran-stimulated cultures, has little, if any, stimulatory effect on TNP-Ficoll-mediated responses. Moreover, Th2 supernatant inhibited the IL-5-enhanced Ig-secretory response stimulated by TNP-Ficoll. This inhibitory effect was due,

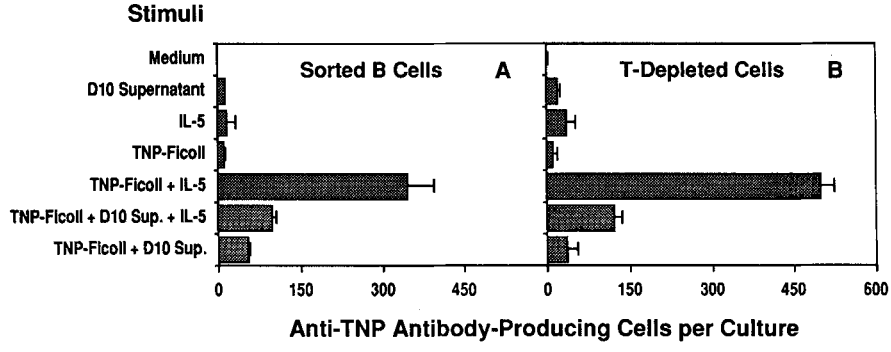

Figure 2. TNP-Ficoll-induced response of sorted B cells stimulated in the presence of D10 supernatant. Splenic B cells were T depleted, and high density, resting B cells were obtained by fractionation over Percoll gradients; some of these cells were stained with phycoerythrin-anti-IgM, and IgM^+ cells were sorted. Sorted B cells (*A*) or T-depleted, high density, splenic cells (*B*) (5×10^6) were stimulated for 4 days with the reagents indicated. D10 supernatant was used at 20%, IL-5 at 20 U/ml, and TNP-Ficoll at 10 ng/ml. The numbers represent the mean ± SD of triplicate cultures.

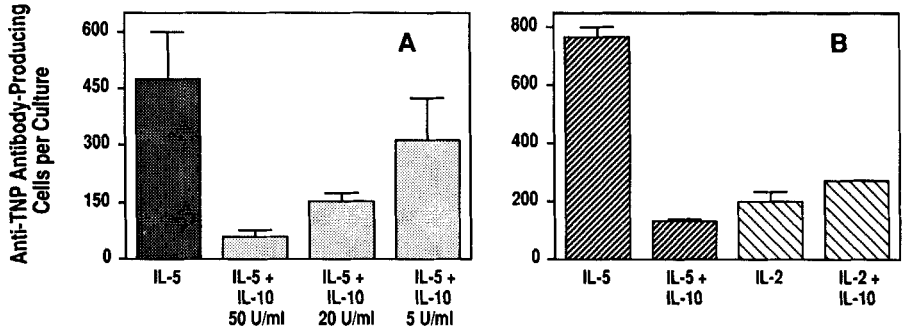

Figure 3. Stimulation of cells with TNP-Ficoll in the presence of IL-10. *A*, resting B cells (7.5×10^5) were stimulated with TNP-Ficoll (10 μg/ml) for 4 days. Some cultures received IL-5 (20 U/ml) and either mock supernatant or the indicated doses of IL-10, either alone or in combination. The number of PFC was measured after 4 days of culture. Cultures stimulated with TNP-Ficoll induced no detectable PFC, and cultures stimulated with lymphokines only, in the absence of TNP-Ficoll, induced <26 PFC/culture. Results are expressed as mean ± SD of triplicate cultures. *B*, resting B cells (10^6) were stimulated as in *A*. Cultures received either IL-5 (20 U/ml) or IL-2 (100 U/ml). IL-10 (100 U/ml) was added to some cultures. Anti-TNP PFC were measured after 4 days of culture. Cultures stimulated with TNP-Ficoll, IL-2, IL-5, or IL-10 alone presented 26, 60, 54, and 2 PFC, respectively.

TABLE III

IL-10-mediated inhibition of IL-5- but not IL-2-mediated Ig production stimulated by anti-δ-dextran[a]

Stimuli	IgM (ng/ml)		
	Medium	IL-10 (100 U/ml)	IL-10 (25 U/ml)
Medium	<5	<5	<5
Anti-δ-dextran	170	<5	45
IL-5	100	<5	<5
Anti-δ-dextran + IL-5	22,550	1,180	4,790
IL-2	<5	<5	<5
Anti-δ-dextran + IL-2	3,520	2,805	4,420

[a] Resting B cells (2.5×10^4) were stimulated with the reagents indicated, and the IgM levels in the supernatants harvested after 6 days of culture were determined by ELISA. Anti-δ-dextran was used at 10 ng/ml, IL-5 was used at 50 U/ml, and IL-2 was used at 150 U/ml.

TABLE IV

Proliferation induced by anti-δ-dextran in the presence of IL-10[a]

Stimuli	[^{3}H]TdR Incorporation (cpm)	
	Medium	IL-10 (25 U/ml)
Anti-δ-dextran	180,466	157,713
Anti-δ-dextran + IL-2	182,093	162,015
Anti-δ-dextran + IL-5	206,988	183,818
Medium	3,131	3,425

[a] Resting B cells (2×10^5) were stimulated for 2 days with the indicated reagents. Proliferation was measured by [^{3}H]TdR incorporation. IL-2 was used at 50 U/ml, IL-5 was used at 50 U/ml, and anti-δ-dextran was used at 10 ng/ml. Results represent the arithmetic mean of triplicate cultures. SD was <10% in all groups.

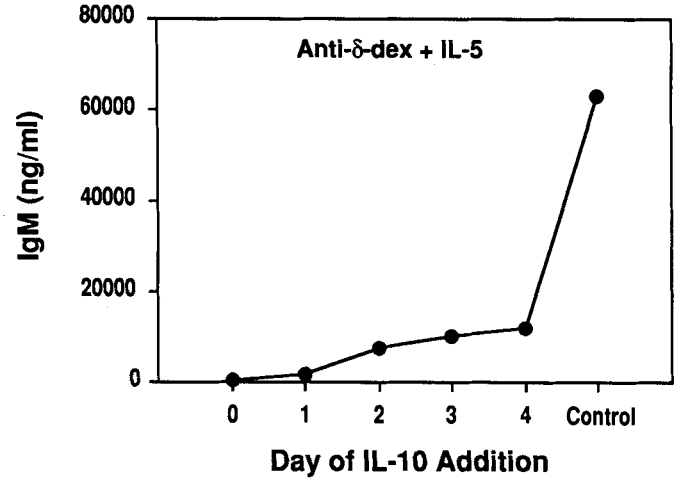

Figure 4. Kinetics of the addition of IL-10. Resting B cells (2.5×10^4) were stimulated with anti-δ-dextran (10 ng/ml) and IL-5 (75 U/ml). IL-10 (25 U/ml) was added at the indicated periods after the initiation of cultures, and the supernatants were harvested after 6 days of stimulation. Control cultures were stimulated with anti-δ-dextran, IL-5, and supernatants from mock-transfected cells at day 0; IgM levels were measured by ELISA.

in part, to IL-10, because it was partially reversed by the addition of anti-IL-10 antibody and because IL-10 inhibited the IL-5-enhanced TNP-Ficoll response. The fact that D10-mediated inhibition of IL-5-enhanced TNP-Ficoll responses was seen even when IgM$^+$ sorted B cells were used indicates that the IL-10-mediated effect is at the level of the B cell. This extends the observations of others who found that IL-10 inhibits both macrophage- and NK-mediated activities (26, 28) (M. Howard, personal communication). The reported finding that IL-10 supports B cell viability (29), coupled with our findings that IL-10 affects neither anti-Ig-stimulated B cell proliferation nor Ig secretion stimulated by IL-2 and either anti-δ-dextran or TNP-Ficoll, makes it unlikely that the observed inhibition reflects a generalized suppressive effect on B cell function.

The finding that D10 supernatant stimulates Ig secretion by anti-δ-dextran-stimulated B cells but not by TNP-Ficoll-stimulated B cells (even though IL-10 inhibits both responses) can be explained by the different culture requirements for these two stimuli. There is a requirement of a high B cell density for elicitation of optimal anti-TNP antibody production and of a 20-fold lower cell concentration for responses to anti-δ-dextran-mediated stimulation. In support of this, we observed that Ig production stimulated by anti-δ-dextran in the presence of D10 supernatant was significantly less when the cell density of the cultures was increased and, conversely, D10 became increasingly stimulatory for TNP-Ficoll responses when cells were cultured at a lower cell density (data not shown). Although the D10 supernatant-mediated inhibition is cell density dependent, the IL-10-mediated inhibition is not. Preliminary data suggest that the D10 supernatant contains lymphokines that can overcome the inhibitory effect of IL-10, but this effect is seen only at low cell density.

The absence of IL-10-mediated inhibition of responses stimulated by TNP-Ficoll or anti-δ-dextran in the presence of IL-2 suggests that the suppressive effect of IL-10

TABLE V

Effect of the addition of anti-IFN-γ and rIFN-γ on the response stimulated by TNP-Ficoll and anti-δ-dextran[a]

Stimuli	% of Control Response	
	Control Antibody	Anti-IFN-γ
TNP-Ficoll + IL-5	100	102
Anti-δ-dextran + IL-5	100	127

Stimuli	% of Control Response		
	Medium	IFN-γ (1 U/ml)	IFN-γ (10 U/ml)
TNP-Ficoll + IL-5	100	82	56
TNP-Ficoll + IL-5 + IL-10	2	1	2
Anti-δ-dextran + IL-5	100	45	11
Anti-δ-dextran + IL-5 + 10	3	3	2

[a] Resting B cells were stimulated with either TNP-Ficoll (10 ng/ml) (7×10^5 cells/culture) or anti-δ-dextran (10 ng/ml) (2.5×10^4 cells/culture). The number of anti-TNP antibody-producing cells in cultures stimulated with TNP-Ficoll was measured after 4 days of culture. Cultures stimulated with TNP-Ficoll plus IL-5 (20 U/ml) presented 520 PFC. IgM levels in the supernatants of cultures stimulated with anti-δ-dextran (10 ng/ml) were measured after 6 days of culture. Stimulation with anti-δ-dextran plus IL-5 (50 U/ml) induced 2675 ng/ml IgM. Stimulation of cultures with TNP-Ficoll alone induced 74 PFC, and stimulation with IL-5 alone induced 90 PFC. Stimulation with either anti-δ-dextran or IL-5 induced <5 ng/ml IgM.

on IL-5 is selective. The fact that IL-10 suppresses IL-5-mediated B cell differentiation to Ig-secretory cells, but not B cell proliferation, raises the possibility that it affects primarily the late steps of B cell differentiation, rather than earlier steps, which favor B cell growth and expansion. This possibility is supported by the observation that IL-10 has a stimulatory effect on an early step of B cell activation, as reflected by its ability to increase expression of MHC class II molecules and B cell viability (29). The finding that IL-10 suppressed Ig secretion by >60% even when added as late as day 4 in a 6-day culture also supports the idea that it is inhibiting a late stage of B cell activation. The selective action of IL-10 has also been recently reported by de Waal Malefyt et al. (30), who demonstrated that IL-10 mediates a marked reduction in LPS-induced secretion of IL-1α, TNF-α, granulocyte/macrophage-CSF, and granulocyte-CSF by human monocytes and a much lesser inhibition of IL-6, IL-1β, and IL-8 secretion.

The fact that a composite of lymphokines secreted by a clone of T cells (e.g., Th2-derived lymphokines) contains both B cell-stimulatory and -inhibitory activities is not unprecedented. Thus, a Th1-secreted product, IFN-γ, has been shown to mediate inhibition of several B cell functions (31, 32), whereas another Th1-derived cytokine, IL-2, is stimulatory (33–35). Likewise, the Th2-secreted product IL-4 mediates a number of B cell-inhibitory activities (36–38), whereas IL-5 mediates stimulatory activities (4, 34, 35, 39).

These data, in conjunction with the observation that activated B cells secrete IL-10, raise the possibility that IL-10 may serve the function of suppressing bystander-mediated B cell activation stimulated by autoantigens or lymphokines. Thus, high local concentrations of IL-2, which can be achieved during an Ag-specific response by cognate T cell-mediated help, would serve to override this IL-10-mediated inhibition. Sorting out the conditions under which stimulation or inhibition is observed with these cytokines may reveal additional diversity in Ag-stimulated pathways of B cell activation.

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