

T CELL-DERIVED B CELL DIFFERENTIATION FACTOR(S)

Effect on the Isotype Switch of Murine B Cells*

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Small IgM⁺, IgD⁺ B cells can switch to the production of other classes (and subclasses) of immunoglobulin (Ig) (1–4). This switch can be induced by either antigenic (5, 6) or mitogenic stimulation (7). The nature of the antigen (8), the site of antigen injection (9), and the nature of the T cell response (8, 10–13) can determine the class of Ig produced in vivo. In particular, the switch in isotype synthesis is relatively T cell dependent (8, 10, 11). Furthermore, T cells can influence the subclass of IgG secreted by B cells (12, 13). However, in low density cell cultures in the presence of lipopolysaccharide (LPS),¹ IgG secretion by murine B cells may not require T cells (5, 14–17).

There is mounting evidence that T cell-derived factors can profoundly influence the growth and maturation of lymphoid cells (18, 19). These factors can be obtained from T cell hybridomas, lines, and clones. There are reports (20–25) indicating that subsets of T cells can give isotype specific help, i.e., can enhance the synthesis of a particular isotype. It was of interest, therefore, to determine whether T cell lines and hybridomas produced factors that affected the isotype produced by B cells.

In the studies reported here we have used B cells stimulated by LPS to investigate the effect of several T cell-derived supernatants on Ig isotype secretion. Our results suggest the existence of a T cell lymphokine(s) produced by a T cell hybridoma and two T cell lines which can induce B cell differentiation. In the presence of LPS, this B cell differentiation factor(s) (BCDF)² enhances IgG secretion by surface immunoglobulin (sIgG⁺) cells and, in particular, substantially increases the secretion of IgG₁.

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¹ *Abbreviations used in this paper:* AEF, allogeneic effect factor; BCDF, T cell-derived B cell differentiation factor; C', complement; CAS, supernatant from concanavalin A-activated spleen cells; Con A, concanavalin A; CSF, colony-stimulating factor; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HCSF, histamine colony-stimulating factor; IL-1, IL-2, interleukin 1 and 2; LPS, lipopolysaccharide; MAF, macrophage-activation factor; NRIG, normal rabbit Ig; PBS, phosphate-buffered saline; PFC, plaque-forming cell(s); R α δ , rabbit anti- δ chain; R α μ , rabbit anti- μ chain; R α γ , rabbit anti-mouse γ ; RAMIg, rabbit anti-mouse Ig; RIA, radioimmunoassay; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; sIg, surface immunoglobulin; TRF, T cell-replacing factor(s).

² We have previously reported the induction of polyclonal IgM secretion by supernatants from T cell lines and hybridomas (26). Since these T cells also secrete TRF, we previously referred to this polyclonal activity as TRF. We have recently shown that T cell lines that do not secrete conventional TRF³ are capable of inducing polyclonal IgM secretion. Thus, we now refer to this activity as B cell differentiation factor for IgM (BCDF_M). The relationship between BCDF_M and the BCDF defined in the present paper, which enhances polyclonal IgG secretion (BCDF_G), is not clear. Therefore, in this report BCDF will be used to describe T cell-derived factors that can effect polyclonal Ig secretion of either isotype.

BCDF appears to be different from other known lymphokines, such as interleukin 1 (IL-1), interleukin 2 (IL-2), T cell-replacing factor (TRF), some colony-stimulating factors (CSF), macrophage-activation factors (MAF), and immune interferon (IFN- γ).

Materials and Methods

Animals. Female BALB/c mice (Cumberland Farms, Clinton, TN) were used at 8–16 wk of age.

Deletion of T Cells. B cells were prepared by treatment of spleen cell suspensions with a monoclonal anti-Thy-1.2 (HO-13.4) and baby rabbit complement (C') (Pel-Freeze Biologicals, Rogers, AR). Alternatively, spleen cells were first treated with a combination of rat hybridoma antibodies against Thy-1.2 (HO12.4), LyT-1 (53.7.313), and LyT-2 (52.6.72), followed by rabbit anti-mouse γ chain and C', as described (26). These treatments abolished the proliferative response to concanavalin A (Con A).

Culture Conditions. BALB/c B cells were cultured in flat-bottomed microtiter plates (Linbro Scientific Co., Hamden, CT) at 0.5×10^5 – 5×10^5 cells/ml in RPMI 1640 with penicillin, streptomycin, and gentamycin (10 μ g/ml), 50 μ M 2-mercaptoethanol, and 10% fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, NY). Cultures were incubated in a humidified atmosphere of 83% N₂, 10% CO₂, 7% O₂ at 37°C.

Plaque-Forming Cell (PFC) Assay. Enumeration of Ig-secreting cells was accomplished by the reverse plaque assay of Gronowicz et al. (27). The antiserum used for detecting IgM-secreting cells has been described (28). The anti- γ serum was raised against free gamma chain (29) and did not develop plaques with IgM-secreting myelomas.

Antibodies. The preparation of affinity-purified rabbit antibodies against mouse Ig (RAMIg), μ (R μ), and γ (R γ) heavy chains, and the hybridoma antibody, anti- δ^s (H10.4.22) has been described (30). The anti- γ reacts with all four subclasses of IgG as determined by binding of the relevant myeloma proteins. Two monoclonal anti-Thy 1.2 antibodies were used (31); the mouse IgM κ product of HO-13.4 hybridoma cells was obtained from the Cell Distribution Center of the Salk Institute (La Jolla, CA), and the rat IgG product of HO-12.4 hybridoma cells was obtained from Dr. Noel Warner (Becton, Dickinson & Co., Palo Alto, CA). The rat anti-Lyt-1 and anti-Lyt-2 hybridomas (31) were also obtained from the Cell Distribution Center of the Salk Institute. The purification of the monoclonal anti-Lyt-1 and anti-Lyt-2 antibodies has been described previously (26). Fluorescein-isothiocyanate-conjugated F(ab')₂-rabbit anti-mouse γ (FITC-F(ab')₂RAM γ) has been described (32).

Cell Sorting. BALB/c splenocytes were stained with FITC-F(ab')₂-R α γ (67 μ g/ml) as described (32). This reagent stains ~5% of spleen cells. Stained cells were sorted sterilely on a FACS III (B-D FACS System, Becton, Dickinson & Co., Sunnyvale, CA). Scatter gates were set to exclude dead cells and erythrocytes. The brightest 15–20% cells were sorted from the negative population. After sorting, the cells were reanalyzed to determine the percentage of positive cells remaining in the negatively selected population. Typically <1% of the negatively sorted cells were positive. Sorted cells were pelleted and suspended in medium and treated with monoclonal anti-Thy-1.2 and C'. In some experiments, T cells were killed before sorting; similar results were obtained with each of the two procedures. Sorted cells, along with stained unsorted cells or untreated cells, were then cultured as described above.

T Cell Supernatants (Table I)

HYBRIDOMA FS7-6.18 CELLS (33). These were obtained from Dr. P. Marrack, and were pulsed with Con A, and the supernatants prepared as previously described (26).

T CELL LINES PK 7.1.1a AND PK 7.1.2. Secondary alloreactive AKR anti-C57BL/6 (B6) cells (10⁴/ml) were subcultured weekly in the presence of irradiated (3,300 rad) B6 stimulator cells (10⁶/ml) in medium supplemented with rat spleen Con A supernatant (CAS) as a source of IL-2 (34). These cells have been maintained in culture (75 cm² Falcon bottles, flat-bottomed, in 20–40 ml medium [Falcon Labware, Oxnard, CA]) for >1 yr (34). Lymphokine release was induced after washing the cells in IL-2-free medium containing 2% FCS, pulsing them for 3 h with 10 μ g/ml Con A, washing, and culturing for 24 h in medium free of both IL-2 and Con

TABLE I
Lymphokines Produced by T Cell Hybridomas and Long-Term Lines

Cell designation	Lymphokine										Reference
	IL-2	TRF	MAF	IFN- γ	CSF				HCSF	BCDF	
					GM	MEG	EO	E			
PK 7.1.1a	-	-	ND*	-	+	+	+	+	+	+	34
PK 7.1.2	-	-	ND	-	+	+	+	+	+	+	34
FS7-6.18	+	-	ND	ND	ND	ND	ND	ND	ND	+	33
PC-AKR clone 29	-	-	+	+	+	+	-	-	-	-	34, 35
PC-AKR clone 96	-	-	+	+	-	-	-	-	-	-	34, 35
B151K12	-	+	ND	ND	ND	ND	ND	ND	ND	-	36
C.C3.11.75	-	+	ND	ND	ND	ND	ND	ND	ND	-	38, 39

* Not determined.

A. The supernatants from these cells have been shown to lack IL-2 and TRF, but to contain CSF and histamine-producing cell stimulating factor (HCSF) (See Table I) (34).

Radioimmunoassay (RIA) for Secreted Ig. Supernatants from 6-d cultures of stimulated cells were assayed for the presence of secreted IgM and IgG by a solid phase RIA as previously described (28). Microtiter plates (Cooke Engineering, Alexandria, VA) were coated with affinity-purified RAMIg and 1% FCS. IgM was detected with [125 I]R $\alpha\mu$ and IgG with [125 I]R $\alpha\gamma$. These reagents had negligible cross-reactivity (<1%) with inappropriate heavy chains or light chains. For quantitation, standard curves using purified myeloma IgM (MOPC-104E) or mouse IgG were included in each assay.

Radiolabeling of LPS-treated B Cells. B cells were cultured at 2×10^5 /ml in flat-bottomed 75-cm 2 flasks (Falcon Labware) with 20 μ g/ml LPS (*Salmonella typhosa*, Difco Laboratories, Detroit, MI) and the appropriate concentration of T cell supernatant. After 6 d cells were harvested and washed with balanced salt solution. 0.3×10^7 – 1×10^7 cells were then labeled with [3 H]leucine for 6 h as previously described (40). Secreted material was dialyzed against PBS before immunoprecipitation or column chromatography.

Immunoprecipitation and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Secreted material (3 H) or samples from protein A-Sepharose columns (3 H or 125 I) were treated with saturating amounts of affinity-purified rabbit antibodies directed against mouse μ , γ chain or ovalbumin (control). Complexes were bound to fixed *S. aureus*. The bacteria were washed with PBS, and the adherent radioactive material was eluted by boiling in 1% SDS containing 2-mercaptoethanol. Samples of the eluate were electrophoresed on 7.5% SDS-PAGE. The areas under the μ and γ chain peaks were then determined from the gel plots.

Separation of IgG Subclasses by Protein A Column Chromatography. To identify subclasses of IgG secreted by [3 H]leucine-labeled cells, a modification of the method described by Ey et al. (37) was used. Briefly, columns containing 2 ml of Sepharose-protein A (1 mg protein A [Pharmacia Fine Chemicals, Piscataway, NJ] per ml Sepharose) were washed with 50 ml of PBS pH 8.0 (PBS $_8$). Aliquots (1–2 ml) of [3 H]leucine-labeled material, dialyzed into PBS $_8$, were passed over the column, and the pH 8.0 fall through and wash were collected. The adherent material was eluted stepwise with 10 ml PBS-pH 7.0, 0.1 M Na citrate, pH 6.0 and Na citrate, pH 5.0. The fall through and the fractions eluting at each pH were dialyzed for 16 h at 4°C against PBS, pH 7.3 and the Ig immunoprecipitated from the dialyzed samples as described above.

Separate experiments were performed to determine the elution profiles of each IgG subclass and established that 125 I-IgG $_1$ (MOPC-21), 125 I-IgG $_{2a}$ (RPC-5), 125 I-IgG $_{2b}$ (MOPC-195), and 125 I-IgG $_3$ (J606) eluted from the protein A Sepharose column at pH 8.0 (γ_1), 6.0 (γ_{2a}), and 5.0 (γ_{2b} and γ_3), respectively. Moreover, it was found that the IgG eluted at pH 8.0 was not bound by *S. aureus* (at pH 7.3), whereas the IgG eluted at pH 6.0 and 5.0 were bound. This further confirmed that the pH 8.0 eluate contained IgG $_1$, whereas the pH 6.0 and pH 5.0 eluates

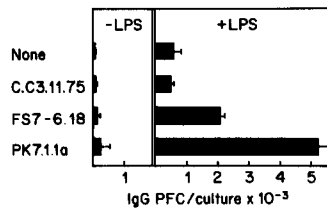


FIG. 1. Effect of T cell supernatants on IgG production by B cells. BALB/c B cells were cultured at 2×10^5 /ml (4×10^4 /well) for 6 d, then assayed for IgG-secreting cells by the reverse plaque assay. T cell supernatants were used at the following concentration (vol/vol): FS7-6.18 supernatant 25%; PK 7.1.1a supernatant 2%; C.C3.11.75 supernatant 5%.

contained IgG_{2a}, IgG_{2b}, and IgG₃, respectively. Because IgG_{2b} and IgG₃ could not be separated by chromatography on protein A-Sepharose, the pH 5.0 eluate will be referred to as IgG_{2b}/IgG₃. In terms of the [³H]leucine-labeled Ig, 80–90% of [³H]leucine labeled IgM eluted at pH 8.0, whereas the remainder eluted at pH 5–7. It is of interest that the small amount of IgM eluting with IgG_{2b}/IgG₃ at pH 5.0 (~10%) bound to *S. aureus*, whereas that eluting with IgG₁ (pH 8.0) did not.

Results

Effect of Con A-Induced Supernatant from FS7-6.18 and PK 7.1.1a on Ig Production by B Cells. We determined whether supernatant from the T cell hybridoma FS7-6.18 and the T cell lines PK 7.1.1a and PK 7.1.2 could enhance the LPS-induced IgG production by splenic B cells in low density cultures. It was desirable to remove T cells to facilitate interpretation of the results because some supernatants tested contained lymphokines that could activate T cells; such activated T cells might influence the B cell response. Therefore, T cells were depleted by treatment with either anti-Thy-1.2 plus C', or anti-Thy-1.2 plus anti-Lyt-1 and anti-Lyt-2 followed by rabbit anti-γ and C'. The results with both B cell populations were similar. As shown in Fig. 1, LPS induced IgG PFC in B cells cultured for 6 d. Supernatants from both FS7-6.18 and PK 7.1.1a markedly enhanced the LPS-induced IgG response, whereas neither supernatant appeared to elicit a significant IgG response in the absence of LPS (see below). Supernatants from line PK 7.1.2 had activity identical to that found in PK 7.1.1a supernatant and have been used in similar experiments; for clarity, only results obtained with PK 7.1.1a supernatants are presented. In contrast, supernatants from C.C3.11.75 (38–39), a T cell line, and B151K12 (36), a T cell hybridoma (both of which produce TRF and induce B cells to secrete IgM) had no effect on IgG secretion (Fig. 1 and data not shown). This suggests that a conventionally defined³ TRF is not responsible for the enhancement of the IgG response in the presence of LPS. Supernatants from several other T cell lines and clones tested in this system (see Table 1) were also found to be negative: BW5147 thymoma (one parent of FS7-6.18), T33F6 hybridoma described by Pacifico and Capra (42), PC-AKR CL-96, which produces MAF and IFN-γ (34, 35), and PC-AKR CL-29, which produces IFN-γ, MAF, and several CSF (34). CAS uniformly suppressed the LPS-induced IgG PFC. Thus, IgG production was enhanced by supernatants from two T cell lines and one T cell hybridoma, and the activity of these supernatants did not correlate with the presence of TRF, IL-2, MAF, GM-CSF, or Meg-CSF (see Table I).

³ TRF is used here to denote a factor that can replace T cells in the in vitro response to sheep erythrocytes, as defined by Schimpl and Wecker (47).

Effect of Supernatant Concentration and Cell Density on IgG Secretion. The relative potency of the two positive supernatants (FS7-6.18 and 7.1.1a) was assessed (Fig. 2). The FS7-6.18 supernatant, at any concentration tested, only induced IgG PFC in the presence of LPS. PFC were increased approximately fourfold as compared with PFC from cultures treated with LPS alone (Fig. 2). The PK 7.1.1a supernatant was >10-fold more potent than FS7-6.18 in enhancing an LPS response, although maximum responses obtained with each supernatant were similar. Although FS7-6.18 did not increase the LPS-induced IgM response at any cell density tested (Fig. 3), IgG secretion was substantially enhanced at a cell number as low as 1×10^5 cells/ml. At cell densities below 10^5 cells/ml, an abrupt decline in the IgG response occurred. This may reflect a requirement for accessory cells in the responder cell population. Analogous results were obtained with PK 7.1.1a supernatant (data not shown). The data in Fig. 3 reflect the total IgG secreted over the 6-d culture period determined in a RIA; similar results were obtained in a PFC assay performed on day 6 of culture (data not shown).

T Cell Supernatants Increase the Frequency of IgG-secreting Cells. One possible explanation for the enhanced IgG response induced by the T cell supernatants would be that such supernatants simply increased the number of cells recovered at the end of the culture period. This was an important consideration because the FS7-6.18 often

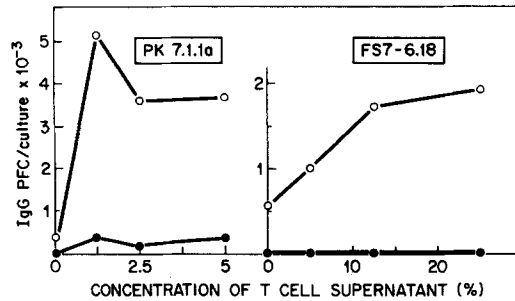


FIG. 2. Effect of T cell supernatant concentration on induction of IgG secretion. BALB/c B cells were cultured at 2×10^6 /ml for 6 d and assayed for IgG secreting cells. Concentration of T cell supernatant is (vol/vol). Data for the PK 7.1.1a and FS7-6.18 supernatants were obtained in separate experiments. ●, without LPS; ○, with LPS.

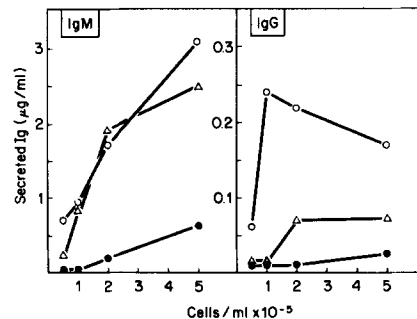


FIG. 3. Effect of cell density on IgG secretion. BALB/c B cells were cultured for 6 d at the indicated initial cell densities. Supernatants were assayed for IgM and IgG by solid phase radioimmunoassay. Concentration of FS7-6.18 supernatant was 25% (vol/vol). ●, control; △, LPS; ○, LPS + FS7-6.18.

enhanced the recovery of viable cells. Thus, the number of viable cells recovered after 6 d was determined and used to calculate the PFC frequency, i.e., number of IgG PFC per 1,000 recovered cells. As shown in Table II, both FS7-6.18 and PK 7.1.1a markedly increased the frequency of IgG secreting cells, and therefore both supernatants appear to induce differentiation rather than simply increase recovery of viable cells.

T Cell Supernatants Induce IgG Secretion by IgG⁻ Cells. The experiments presented above suggest that T cell factor(s) from two sources can enhance the appearance of IgG-secreting cells. In order to determine whether these factors selectively induced differentiation of IgG-bearing cells or induced a switch in IgG⁻ cells, we removed IgG-bearing cells before culture. This was accomplished using the fluorescence-activated cell sorter (FACS). Spleen cells were stained with fluoresceinated F(ab')₂-rabbit anti-γ then analyzed and sorted. This reagent typically stains 5% of BALB/c spleen cells. Gates were set such that the 15–20% most positively stained cells were excluded from the negatively selected population. Thus, even weakly positive cells were removed by this procedure. The positively selected cells were not cultured for technical reasons. Negatively selected cells were then reanalyzed on the FACS and cultured in the usual manner; untreated cells and/or stained but unsorted cells were also cultured as controls. A typical experiment is shown in Fig. 4. It is apparent that the removal of IgG-positive cells has no effect on the IgG-response of the cells to LPS or LPS plus T cell factors. Results similar to those shown in Fig. 4 were obtained in three other experiments.

We also quantitated the amount of radioactive IgG secreted by both sorted and unsorted radiolabeled cells. Cells were labeled with [³H]leucine, and the secreted material was analyzed by immunoprecipitation with rabbit anti-γ followed by SDS-PAGE. A typical gel profile is shown in Fig. 5. Similar cell numbers secreting identical acid-precipitable counts were used so that direct comparisons could be made. It is obvious that virtually identical amounts of IgG were secreted by both sorted and unsorted cells. In addition, the secreted material from four cultures (two separate experiments) was precipitated with RAMIg, and the quantity of secreted IgM and IgG was determined by calculating the areas under the H chain peaks of the SDS gels. Although some variability in the total amount of IgG secreted was apparent, sIgG⁻ cells did not secrete substantially less IgG than unsorted cells (4.4% decrease in IgG secretion by sIgG⁻ cells).

TABLE II
Effect of T Cell Supernatants on the Frequency of LPS-induced IgG-secreting Cells

Experiment	Initial cell density × 10 ⁵ /ml	Addition to culture*		
		None	FS7-6.18	PK 7.1.1a
1	2	0.8‡	7.8	ND§
2	2	0.3	19.2	ND
3	1	2.0	9.0	24.0

* See Legend to Fig. 1 for experimental details.

‡ IgG PFC per 1,000 cells recovered.

§ Not done.

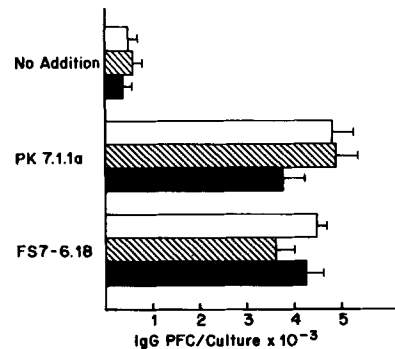


FIG. 4. Induction of IgG secretion in IgG⁻ cells cultured with T cell supernatants in the presence of LPS. BALB/c B cells (prepared by treatment with anti-Thy-1.2 + C') were stained with FITC-F(ab')₂-Rαγ and positively staining cells were sorted away as described in Materials and Methods. Three groups of cells were then cultured in microtiter plates at 2×10^5 /ml with 20 μg/ml LPS: □, untreated cells; ▨, cells which were stained but not sorted; and ■, sorted (IgG-negative) cells. After 6 d cultures were assayed for IgG PFC.

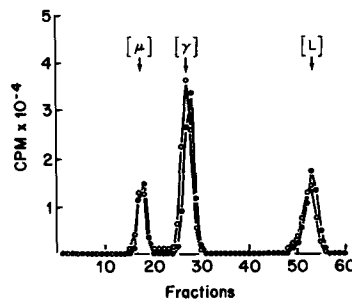


FIG. 5. Subclass analysis of the IgG secreted by cultured B cells. IgG positive cells were sorted away as described in Materials and Methods and the legend to Fig. 4. Unsorted (●) and sorted (○), IgG negative cell populations were then cultured in 10-ml flasks at 2×10^5 /ml for 6 d with LPS and FS7-6.18 supernatant (20%). Equal numbers of cells (3×10^6) were labeled with [³H]leucine and the secreted material (which had equal amounts of TCA-precipitable counts) was immunoprecipitated with rabbit anti-γ and *S. aureus*. After elution, samples were electrophoresed under reducing conditions on 7.5% SDS-polyacrylamide gels. The IgM (μ chain) shown in the gel was also bound by NRIg and *S. aureus* (not shown) and does not indicate the presence of anti-μ or anti-L antibodies in the anti-γ serum. A portion of the IgG also bound directly to *S. aureus* (not shown) and reflects the presence of protein A-binding IgG₃/IgG_{2b}.

Furthermore, as shown in Table III, a comparison of the amount of secreted IgG and IgM showed that: (a) cells treated with LPS alone secrete predominantly IgM (average of 96%), and (b) addition of either FS7-6.18 or PK 7.1.1a supernatant resulted in a striking increase in IgG secretion (average of 20 and 24% of the total Ig, respectively). Taken together, these results suggest that the T cell supernatants preferentially enhance the LPS-induced IgG secretion by sIgG⁻ cells.

Subclass Analysis of the IgG Secreted by Cultured B Cells. Since T cells are known to influence the subclass as well as the class of IgG secreted by B cells, we determined whether the T cell supernatants affected the subclass of IgG secreted by cells cultured in the presence of LPS. To quantitatively analyze the subclass of IgG secreted in the above experiments, we labeled equal numbers of LPS-treated B cells (\pm PK 7.1.1a supernatant) with [³H]leucine on day 6 of culture; comparable amounts of [³H]leucine

TABLE III
Effect of T Cell Supernatants on LPS-induced IgM and IgG Secretion*

LPS plus supernatant from T cell lines added to culture	Class of Ig secreted (percent of total secreted Ig)‡					
	Experiment 1		Experiment 2		Experiment 3	
	IgM	IgG	IgM	IgG	IgM	IgG
LPS	99	1	93	7	ND	ND
LPS + FS7-6.18	87	13	84	16	68	32
LPS + PK 7.1.1a	83	17	79	21	65	35

* 1×10^6 – 2×10^6 cells were cultured at 2×10^5 /ml for 6 d, then labeled with [3 H]leucine. Secreted material was analyzed by immunoprecipitation with RAMIg and *S. aureus*, and SDS-PAGE as described in Materials and Methods.

‡ Calculated from μ and γ peaks on SDS-PAGE.

TABLE IV
Effect of T Cell Supernatants on the IgG Subclasses Produced by LPS-treated B Cells

Experiment	LPS plus supernatant from T cell lines added to culture	IgG subclass secreted (percent of total IgG)*		Increase in IgG subclass‡	
		IgG ₁	IgG ₃ /IgG _{2b}	IgG ₁	IgG ₃ /IgG _{2b}
1	O	30	70	—	—
	FS7-6.18	85	15	4	1
	PK 7.1.1a	97	3	14	1
2	O	46	54	—	—
	FS7-6.18	97	3	38	1
	PK 7.1.1a	93	7	19	1

* Based on the area under the γ_1 or γ_3/γ_{2b} heavy chain peaks on SDS-PAGE.

‡ cpm of γ_1 or γ_3/γ_{2b} chain of secreted IgG on SDS-PAGE of LPS + supernatant/LPS alone.

were incorporated into total acid-precipitable material and into secreted Ig in both cultures.

In experiments shown in Table III, we observed that $\leq 7\%$ of the Ig secreted by LPS-stimulated B cells was IgG. Approximately 50% of this IgG could not be bound by *S. aureus* at neutral pH, suggesting that it was IgG₁ (data not shown). In contrast, in cells cultured in the presence of LPS plus PK 7.1.1a supernatant, 20–25% of the Ig secreted was IgG (Table III). Little or none of this IgG was bound to *S. aureus* (data not shown). To further confirm that the increase in IgG observed in B cells cultured in the presence of LPS plus PK 7.1.1a was IgG₁, the subclasses of IgG in the secreted material were analyzed by chromatography on protein A-Sepharose (to separate IgG₁, IgG_{2a}, and IgG_{2b}/IgG₃ [37]). The column fractions were then analyzed by immunoprecipitation and SDS-PAGE. The results of two experiments are shown in Table IV. LPS-treated cells secrete predominantly IgG_{2b}/IgG₃ with lesser amounts of IgG₁; IgG_{2a} was not detectable. In contrast, supernatants from FS7-6.18 or PK 7.1.1a caused a striking shift to IgG₁ production in LPS-treated cells. This is particularly apparent

in the amount of each IgG subclass produced in the absence and presence of T Cell supernatants (Table III). It is evident that the increase in IgG PFC is due mainly to IgG₁-secreting cells, since the amount of secreted IgG_{2b}/IgG₃ is similar in the presence and absence of the T cell supernatants.

Discussion

This paper describes a lymphokine(s) produced by a T cell hybridoma and two alloreactive long-term T cell lines that markedly increases the recovery of IgG-secreting cells after culture of murine B cells in the presence of LPS. We have termed this lymphokine BCDF. Whereas LPS alone induces secretion of small amounts of IgG_{2b}/IgG₃ and IgG₁, the combination of LPS and BCDF induces a substantial increase in IgG₁. The cells producing IgG₁ in the presence of LPS and BCDF were originally sIgG⁻, suggesting that LPS-stimulated B cells are induced to switch in the presence of BCDF.

Secretion of IgG can be induced in murine B cells in vitro by the mitogen, LPS (7, 14-17). The LPS-mediated induction of IgG secretion has been shown to be T independent and optimal at low cell density (7, 15). Our results are entirely consistent with these findings. The fact that IgG secretion is optimal at low initial cell densities suggests that several rounds of cell division may be required to obtain a switch to IgG, and there is evidence to support this contention (43, 44). We found that the addition of supernatants from some T cell lines (FS7-6.18 and PK 7.1.1a), but not others (C.C3.11.75, B.151-K12, BW 5147, T33F6, PC-AKR CL 96, PC-AKR CL 29), could greatly enhance the in vitro IgG response. This enhancement occurred independently of the presence of T cells in the culture and was optimal at low cell density. The enhancement of IgG secretion by the T cell supernatants was dependent on the presence of LPS, suggesting the necessity for a specific signal induced by LPS. This could be a proliferative signal per se, or one that induces receptors for BCDF.

Several lines of evidence indicate that the cells secreting IgG in both antigen and LPS-treated cultures arise from IgM-bearing cells: (a) early (but not late) addition of anti- μ to cultures of normal spleen cells suppresses both IgM and IgG secretion (41, 45, 46); (b) in limiting dilution analysis of LPS-treated B cells, IgG PFC appear in wells that first contain a clone of IgM secreting cells (14); and (c) removal of IgG-bearing cells does not decrease the appearance of IgG secreting cells in LPS-treated cultures (17). Nevertheless, we felt it was essential to prove whether or not BCDF selects and expands B cells already bearing IgG under our experimental conditions. To demonstrate that sIgG⁻ cells were the precursor cells for BCDF-induced IgG secretion, essentially all sIgG⁺ B cells were removed by cell sorting and the sIgG⁻ cells were cultured in the presence of LPS and BCDF. In these experiments, the sIgG⁻ B cells secreted amounts of IgG similar to those secreted by unsorted B cells, indicating that the precursors of the IgG secreting cells were originally sIgG⁻.

The mechanism by which BCDF specifically increases recovery of IgG secreting cells is not known. Two possibilities are: (a) BCDF may selectively expand the B cell population that has been induced to switch to IgG by LPS, thus increasing the number of progeny of a given clone; or (b) BCDF may increase the number of clones that switch. A distinction between these two possibilities may be approached experimentally by determining the effect of BCDF on the IgG precursor frequency using limiting dilution techniques (14). The results of such experiments (Krammer, Isakson,

Pure, and Vitetta, manuscript in preparation) support the contention that BCDF increases the precursor frequency but not the burst size of LPS-induced IgG secreting cells. This finding further suggests that BCDF enhances a switch from IgM to IgG.

Several reports have demonstrated that soluble products of T cells can stimulate B cell differentiation (47, 48). Typically, these T cell factors have been obtained from mixed populations of T cells and their biochemical characterization has remained elusive. Recently, however, the establishment of long-term T cell lines (49–51) has allowed immortalization of particular T cell populations; this has greatly facilitated studies of T cell-derived lymphokines (18, 19, 33, 34). There are several reports of the establishment of T cell lines and hybridomas that secrete products which induce Ig secretion (36, 38, 39). Some of these factors appear to induce Ig secretion alone (26, 52–55) while others may require the presence of antigen (36, 39). Whether these factors are identical or related to each other is not clear; their relationship to conventionally defined TRF is not known. The two we have tested (CC3.11.75 and B151K12) appear to induce predominantly IgM secretion (26).

The mechanisms by which T cells regulate synthesis of particular Ig isotypes are not understood. Ishizaka and co-workers have demonstrated that helper factors specific for IgE can be induced by various stimuli (24, 25). T cells can also influence the IgG subclass secreted by B cells (12, 13). Data from *in vivo* experiments suggest that IgG₃ secretion occurs independently of T cells, whereas IgG₁ secretion is strongly T cell dependent (8, 10). Rosenberg and Chiller (20) showed induction of polyclonal class-specific Ig secretion by several nonspecific stimuli. Whereas some T cell stimuli (e.g., Con A) induced increases in all classes of IgG, others (e.g., complete Freund's adjuvant) induced primarily IgG₁ and IgG₂ secretion. Thus, there is an *in vivo* precedent for our finding that T cells can regulate the subclass of IgG produced by polyclonally activated B cells. Using an *in vitro* system, Augustin et al. (21, 22) have demonstrated that the direct interaction of T cells with polyclonally activated B cells or B cell blasts results in an increase in IgG₁ secretion. In addition, Elson et al. (23) have shown that Con A-activated Peyer's patch cells contain high numbers of T cells that drive LPS-induced B cells specifically to IgA secretion. Taken together, these studies indicate that the secretion of specific classes of Ig can be regulated by T cells or their soluble products.

The mechanism by which B cells switch isotype synthesis is beginning to be understood at the molecular level. Two different types of models have been proposed: (a) switching may be a stochastic process in which cells switch in a random fashion to secretion of previously unexpressed isotypes. The likelihood of productive recombination with a particular C region may, therefore, depend on such factors as distance from the C μ gene, number and type of switch recombination sites (56–59), etc.; (b) switching may be due to the directed induction of a particular isotype. This could be accomplished by isotype specific factors that act at the plasma membrane of B cells and direct particular DNA rearrangements. The two models are not mutually exclusive and there could be both random and isotype directed elements involved in switching. Mongini et al. (13) observed that in the absence of T cells, the magnitude of each isotype response to a T-independent antigen exactly mimicked the 5' to 3' order of the immunoglobulin H-chain genes (μ , γ_3 , γ_1 , γ_{2b} , γ_{2a}). Thus, the likelihood of switching to a given subclass would decrease with increasing distance from the C μ gene. This observation is consistent with a stochastic model. T cells might simply

increase the frequency of switching nonspecifically by driving cell division and/or by increasing the levels of enzymes responsible for switching. In contrast, reports of class specific T cells (20–25) and T cell factors support a model of directed induction of particular isotype switches. The present data do not distinguish between these models. It would be of interest to extend the period of culture to determine whether BCDF can cause switching to Ig isotypes whose C-region genes map further downstream (e.g., IgA). If so, it would indicate that BCDF is not specific in inducing switching to synthesis of a particular isotype.

We have not yet attempted to purify BCDF. Instead, we have relied on a variety of biological assays performed on the BCDF-containing supernatants for the identification of lymphokine activity (Table I). From these comparisons, we conclude that none of the conventionally-defined lymphokines tested, with the possible exceptions of E_o-CSF, E-CSF, or HCSF, mediate this effect. Because the PK lines were derived by allogeneic stimulation, it is possible that they produce allogeneic effect factor (AEF) (60) and that AEF is responsible for the effects we observed. However, AEF is reported to be most effective on cells expressing the haplotype of the stimulator cells (60, 61). In the experiments reported here, BCDF induced IgG secretion by B cells expressing a haplotype (H-2^d) different from that of the stimulator cells (H-2^b). In other experiments (manuscript in preparation), we have shown that BCDF works equally well using strains carrying the H-2^k and H-2^b haplotype. Therefore, although we cannot formally exclude the possibility that BCDF is AEF, we regard this possibility as unlikely. In any case, the lack of an overt correlation with other lymphokines, in particular TRF, suggests that a newly defined T cell factor may be present in the supernatants of these two lines and that this factor affects differentiation of B cells. It should be noted that the two lines (C.C3.11.75 and B.151-K12) that produce a factor which induces polyclonal IgM secretion (26), do not stimulate IgG secretion in this system; thus, BCDF is distinct from this activity as well. The fact that the FS7-6.18 supernatant, which contains interleukin 2 (IL-2) but not TRF (33) can induce IgG secretion, raises the possibility that IL-2 can mediate this effect (even though the PK 7.1.1a and PK 7.1.2 supernatants lack both IL-2 and TRF). In experiments with Dr. S. Gillis (Fred Hutchinson Cancer Center, Seattle, WA), we have found that purified IL-2 has no BCDF activity (data not shown). This strongly suggests that both FS7-6.18 and the PK 7.1.1a and 7.1.2 lines secrete a lymphokine that induces B cell differentiation but that BCDF is not IL-2 or TRF.

Summary

Culturing BALB/c B cells for 6 d at low cell density in the presence of lipopolysaccharide (LPS) results in the appearance of a small number of IgG plaque-forming cells (PFC). The addition of supernatants from concanavalin A (Con A)-induced alloreactive (AKR anti-B6) long-term T cell lines (PK 7.1.1a and 7.1.2) or a T cell hybridoma (FS7-6.18) to LPS-treated B cells resulted in a marked increase in IgG PFC (3–10-fold higher than in cultures treated with LPS alone). The number of induced IgG PFC was not affected by removing IgG-bearing cells on the fluorescence-activated cell sorter, indicating that T cell-derived B cell differentiation factor enhances isotype switching of sIgG⁻ cells, rather than selecting and expanding pre-existing subpopulations of sIgG⁺ cells. We also investigated the subclass of IgG produced in the absence or presence of T cell factors and found that PK 7.1.1a, PK

7.1.2, and FS7-6.18 supernatants selectively increased IgG₁ production. Several other T cell supernatants containing a variety of lymphokines had no effect, suggesting that PK 7.1.1a, PK 7.1.2, and FS7-6.18 lines produce factor(s) that can specifically enhance the recovery of IgG secreting cells in culture in the presence of LPS. These factors, which we have termed B cell differentiation factors, are different from interleukin 1, interleukin 2, T cell-replacing factor, colony-stimulating factor, macrophage-activating factor, and immune interferon. Our results suggest that soluble factors produced by T cell lines and hybridomas can markedly influence both the class and subclass of Ig produced by B cells.

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References

1. Wang, A., S. Wilson, J. Hopper, H. Fudenberg, and A. Nisonoff. 1970. Evidence for control of synthesis of the variable regions of the heavy chains of immunoglobulins G and M by the same gene. *Proc. Natl. Acad. Sci. U. S. A.* **66**:337.
2. Nossal, G. V. J., N. L. Warner, and H. Lewis. 1971. Incidence of cells simultaneously secreting IgM and IgG antibody to sheep erythrocytes. *Cell Immunol.* **2**:41.
3. Pernis, B. 1976. Synthesis of multiple Ig classes by single lymphocytes. *Cold Spring Harbor Symp. Quant. Biol.* **41**:175.
4. Wabl, M. R., L. Forni, and F. Loor. 1978. Switch in immunoglobulin class production observed in single clones of committed lymphocytes. *Science (Wash. D. C.)*. **199**:1978.
5. Press, J. L., and N. R. Klinman. 1973. Monoclonal production of both IgM and IgG₁ antihapten antibody. *J. Exp. Med.* **138**:300.
6. Gearhart, P. J., N. H. Sigal, and N. R. Klinman. 1975. Production of antibodies of identical idiotype but diverse immunoglobulin classes by cells derived from a single stimulated B cell. *Proc. Natl. Acad. Sci. U. S. A.* **72**:1707.
7. Kearney, J. F., and A. R. Lawton. 1975. B lymphocyte differentiation induced by lipopolysaccharide. I. Generation of cells synthesizing four major immunoglobulin classes. *J. Immunol.* **115**:671.
8. Braley-Mullen, H. 1974. Regulatory role of T cells in IgG antibody formation and immune memory to type III pneumococcal polysaccharide. *J. Immunol.* **113**:1909.
9. Gearhart, P. J., and J. J. Cebra. 1979. Differentiated B lymphocytes potential to express particular antibody variable and constant regions depends on site of lymphoid tissue and antigen load. *J. Exp. Med.* **149**:216.
10. Torrigiani, G. 1972. Quantitative estimation of antibody in the immunoglobulin classes of the mouse. II. Thymus-dependence of the different classes. *J. Immunol.* **108**:161.
11. Taylor, R. B. and H. H. Wortis. 1968. Thymus dependence of antibody: Variation with dose of antigen and class of antibody. *Nature (Lond.)*. **220**:927.
12. Slack, J., G. P. der-Balian, M. Nahm, and J. M. Davie. 1980. Subclass restriction of murine antibodies. II. The IgG plaque-forming cell response to thymus-independent Type 1 and Type 2 antigens in normal mice and mice expressing an X-linked immunodeficiency. *J. Exp. Med.* **151**:853.

13. Mongini, P. K. A., K. E. Stein, and W. E. Paul. 1981. T cell regulation of IgG subclass antibody production in response to T-independent antigens. *J. Exp. Med.* **153**:1.
14. Andersson, J., A. Coutinho, and F. Melchers. 1978. The switch from IgM to IgG secretion in single mitogen-stimulated B-cell clones. *J. Exp. Med.* **147**:1744.
15. Andersson, J., A. Coutinho, and F. Melchers. 1978. Stimulation of murine B lymphocytes to IgG synthesis and secretion by the mitogens lipopolysaccharide and lipoprotein and its inhibition by anti-immunoglobulin antibodies. *Eur. J. Immunol.* **8**:336.
16. Kearney, J. F., M. D. Cooper, and A. R. Lawton. 1976. B cell differentiation induced by lipopolysaccharide. IV. Development of immunoglobulin class restriction in precursors of IgG-synthesizing cells. *J. Immunol.* **117**:1567.
17. Gronowicz, E. S., C. Doss, F. Assisi, E. S. Vitetta, R. L. Coffman, and S. Strober. 1979. Surface Ig isotypes on cells responding to lipopolysaccharide by IgM and IgG secretion. *J. Immunol.* **123**:2049.
18. Moller, G., editor. 1980. *Immunol. Rev.* **51**:3-387.
19. Moller, G., editor. 1980. *Immune. Rev.* **54**:5-266.
20. Rosenberg, Y. J., and J. M. Chiller. 1979. Ability of antigen-specific helper cells to effect a class-restricted increase in total Ig-secreting cells in spleen after immunization with the antigen. *J. Exp. Med.* **150**:517.
21. Augustin, A. A., and A. Coutinho. 1980. Specific T helper cells that activate B cells polyclonally. *In vitro* enrichment and cooperative function. *J. Exp. Med.* **151**:575.
22. Martinez-Alonso, C., A. Coutinho, and A. A. Augustin. 1980. Immunoglobulin C-gene expression. I. The commitment to IgG subclass of secretory cells is determined by the quality of the non-specific stimuli. *Eur. J. Immunol.* **10**:698.
23. Elson, C. O., J. A. Heik, and W. Strober. 1979. T cell regulation of murine IgA synthesis. *J. Exp. Med.* **149**:632.
24. Ishizaka, K. 1976. Cellular events in the IgE antibody response. *Adv. Immunol.* **23**:1.
25. Kishimoto, T., and K. Ishizaka. 1975. Immunologic and physicochemical properties of enhancing soluble factors for IgG and IgE antibody responses. *J. Immunol.* **114**:1177.
26. Puré, E., P. C. Isakson, K. Takatsu, T. Hamaoka, S. L. Swain, R. W. Dutton, G. Dennert, J. W. Uhr, and E. S. Vitetta. 1981. Induction of B cell differentiation by T cell factors. I. Stimulation of IgM secretion by products of a T cell hybridoma and a T cell line. *J. Immunol.* **127**:1953.
27. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting Ig of a given type or class. *Eur. J. Immunol.* **6**:588.
28. Isakson, P. C., K. A. Krolick, J. W. Uhr, and E. S. Vitetta. 1980. The effect of anti-immunoglobulin antibodies on the *in vitro* proliferation and differentiation of normal and neoplastic murine B cells. *J. Immunol.* **125**:886.
29. Yuan, D., E. S. Vitetta, and J. R. Kettman. 1977. Cell surface immunoglobulins. XX. Antibody responsiveness of subpopulations of B lymphocytes bearing different isotypes. *J. Exp. Med.* **143**:1055.
30. Puré, E., and E. Vitetta. 1980. The murine B cell response to TNP-polyacrylamide beads: the relationship between the epitope density of the antigen and the requirements for T cell help and surface IgD. *J. Immunol.* **125**:420.
31. Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse differentiation antigens. *Immunol. Rev.* **47**:63.
32. Krolick, K. A., P. C. Isakson, J. W. Uhr, and E. S. Vitetta. 1979. Murine B cell leukemia (BCL₁): organ distribution and kinetics of growth as determined by fluorescence analysis with an anti-idiotypic antibody. *J. Immunol.* **123**:1928.
33. Harwell, L., B. Skidmore, P. Marrack, and J. Kappler. 1980. Concanavalin A-inducible interleukin 2-producing T cell hybridoma. *J. Exp. Med.* **152**:893.
34. Krammer, P. H., M. Dy, L. Hultner, P. Isakson, U. Kees, M.-L. Lohmann-Matthes, F.

- Marcucci, A. Michnay, E. Puré, A. Schimpl, F. Staber, E. Vitetta, and M. Waller. 1981. In Isolation, Characterization and Utilization of T Lymphocytes. G. Fathman and F. Fitch, editors. Academic Press Inc., New York. In press.
35. Marcucci, F., M. Waller, H. Kirchner, and P. H. Krammer. 1981. Production of immune interferon (IFN- γ) by murine T cell clones from long-term cultures. *Nature (Lond.)*. **291**:79.
 36. Takatsu, K., K. Tanaka, A. Tominaga, Y. Kumahara, and T. Hamaoka. 1980. Antigen-induced T cell-replacing factor. III. Establishment of T cell hybrid clone continuously producing TRF and functional analysis of released TRF. *J. Immunol.* **125**:2647.
 37. Ey, P. L., S. J. Prowse, and C. R. Jenkin. 1978. Isolation of pure IgG₁, IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using protein-A-Sepharose. *Immunochemistry*. **15**:429.
 38. Waterfield, J. D., G. Dennert, S. L. Swain, and R. W. Dutton. 1979. Continuously proliferating allospecific T cells. I. Specificity of cooperation with allogeneic B cells in the humoral response to sheep erythrocytes. *J. Exp. Med.* **149**:808.
 39. Swain, S. L., G. Dennert, J. F. Warner, and R. W. Dutton. 1981. Culture supernatants of a stimulated T cell line have helper activity that synergizes with IL-2 in the response of B cells to antigen. *Proc. Natl. Acad. Sci. U. S. A.* **78**:2517.
 40. Vitetta, E. S., S. Baur, and J. W. Uhr. 1971. Cell surface immunoglobulin. II. Isolation and characterization of Ig from mouse splenic lymphocytes. *J. Exp. Med.* **134**:242.
 41. Lawton, A. R., R. Asofsky, M. B. Hylton, and M. D. Cooper. 1972. Suppression of immunoglobulin class synthesis in mice. I. Effects of treatment with antibody to μ -chain. *J. Exp. Med.* **135**:277.
 42. Pacifico, A., and J. D. Capra. 1980. T cell hybrids with arsonate specificity. I. Initial characterization of antigen-specific T cell products that bear a cross-reactive idiotype and determinants encoded by the murine major histocompatibility complex. *J. Exp. Med.* **152**:1289.
 43. Zauderer, M., and B. A. Askonas. 1976. Several proliferative phases precede maturation of IgG-secreting cells in mitogen-stimulated cultures. *Nature (Lond.)*. **260**:611.
 44. Gronowicz, E. S., C. Doss, and J. Schroder. 1979. Activation to IgG secretion by lipopolysaccharide requires several proliferation cycles. *J. Immunol.* **123**:2057.
 45. Pierce, C. W., S. M. Solliday, and R. Asofsky. 1972. Immune responses *in vitro* IV. Suppression of primary IgM, IgG and IgA plaque-forming cell responses in mouse spleen cell cultures by class-specific antibody to mouse immunoglobulins. *J. Exp. Med.* **135**:675.
 46. Kearney, J. F., M. D. Cooper, and A. R. Lawton. 1976. B lymphocyte differentiation induced by lipopolysaccharide. III. Suppression of B cell maturation by anti-mouse immunoglobulin antibodies. *J. Immunol.* **116**:1664.
 47. Schimpl, A., and E. Wecker. 1975. A third signal in B cell activation given by TRF. *Transplant. Rev.* **23**:176.
 48. Dutton, R. W. 1975. Separate signals for the initiation of proliferation and differentiation in the B cell response to antigens. *Transplant. Rev.* **23**:66.
 49. Morgan, D. A., F. W. Ruscetti, and R. Gallo. 1976. Selective *in vitro* growth of T lymphocytes from normal human bone marrows. *Science (Wash. D. C.)*. **193**:1007.
 50. Gillis, S., and K. A. Smith. 1977. Long-term culture of tumor specific cytotoxic T cells. *Nature (Lond.)*. **268**:154.
 51. Sredni, B., H. Tse, and R. H. Schwartz. 1980. Direct cloning and extended culture of antigen-specific MHC-restricted proliferating T cells. *Nature (Lond.)*. **283**:581.
 52. Nabel, G., M. Fresno, A. Chessman, and H. Cantor. 1981. Use of cloned populations of mouse lymphocytes to analyze cellular differentiation. *Cell*. **23**:19.
 53. Nabel, G., J. S. Greenberger, M. A. Sakakeeny, and H. Cantor. 1981. Multiple biologic activities of a cloned induced T cell population. *Proc. Natl. Acad. Sci. U. S. A.* **78**:1157.
 54. Andersson, J., M. H. Schreier, and F. Melchers. 1980. T cell dependent B cell stimulation

- is H-2 restricted and antigen dependent only at the resting B cell level. *Proc. Natl. Acad. Sci. U. S. A.* **77**:1612.
55. Glasebrook, A. L., J. Quintans, L. Eisenberg, and F. Fitch. 1981. Alloreactive cloned T cell lines. II. Polyclonal stimulation of B cells by a cloned helper T cell line. *J. Immunol.* **126**:240.
 56. Nishida, Y., T. Kataoka, N. Ishida, S. Nakai, T. Kishimoto, I. Botcher, and T. Honjo. 1981. Cloning of mouse immunoglobulin E gene and its location within the heavy chain gene cluster. *Proc. Natl. Acad. Sci. U. S. A.* **78**:1581.
 57. Shimizu, A., N. Takahasi, Y. Yamawaki-Kataoka, Y. Nishida, T. Kataoka, and T. Honjo. 1981. Ordering of mouse immunoglobulin heavy chain genes by molecular cloning. *Nature (Lond.)*. **289**:149.
 58. Davis, M. M., S. M. Kim, and L. Hood. 1980. Immunoglobulin class switching: developmentally regulated DNA rearrangements during differentiation. *Cell*. **22**:1.
 59. Kataoka, T., T. Miyata, and T. Honjo. 1981. Repetitive sequences in class-switch recombination regions of immunoglobulin heavy chain genes. *Cell*. **23**:357.
 60. Katz, D. 1977. *Lymphocyte Differentiation, Recognition and Regulation*, Academic Press Inc., New York. 482-529.
 61. Delovitch, T. L., J. Watson, R. Battistella, J. F. Harris, J. Shaw, and V. Paetkau. 1981. In vitro analysis of allogeneic lymphocyte interaction. V. Identification and characterization of two components of allogeneic effect factor, one of which displays H-2 restricted helper activity and the other, T cell growth factor activity. *J. Exp. Med.* **153**:107.