

**SUPPRESSION OF IN VITRO ANTIBODY SYNTHESIS  
BY IMMUNOGLOBULIN-BINDING FACTOR\***

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Stimulation of thymus-derived lymphocytes by antigens or mitogens induces the release of soluble factors which influence the development of antibody-producing cells during a humoral immune response. Such accessory factors may be antigen specific (1-4) or nonspecific (1, 2, 5-7) and may act as amplifiers (1-7) or suppressors (1, 8-10) of the immune response, thus illustrating the distinctive role of activated T cells in the modulation of B-cell responses to antigen (11, 12).

Recently, Fridman and Golstein (13) described a factor from alloantigen-activated T cells which specifically binds to the Fc fragment of IgG (immunoglobulin-binding factor, IBF) and blocks complement (C) activation by IgG (14). Since IBF is synthesized by T cells (13) and since IgG is known to participate in the regulation of B-cell responses (15), it is interesting to speculate on whether IBF might interfere with antibody synthesis. In the present communication, we describe the suppressive effect of IBF on the synthesis of antibodies to T-dependent and T-independent antigens in vitro and submit that IBF may be a soluble mediator of suppressor T cells.

**Materials and Methods**

*Preparation of IBF.* Activated T cells (ATC) were prepared from spleens of 12- to 16-wk old BALB/c mice which had been irradiated with 700 R and injected intravenously with  $10^6$  C3H (4- to 8-wk old) thymus cells 5 days before (16). Supernates containing IBF were collected after 2 h of incubation of  $2 \times 10^6$  ATC which had been separated from non-T cells by removing phagocytic and adherent cells with the aid of carbonyl iron and a powerful magnet, as described in a previous paper (13). From these crude supernates (S-ATC) IBF was isolated on affinity chromatography columns.<sup>1</sup> Briefly, Sepharose 4B beads were activated with cyanogen bromide and coupled with rabbit IgG at a concentration of 5-10 mg/ml. S-ATC at 0°C was then introduced into the columns, which were washed thoroughly with 0.02 M phosphate buffer at pH 7.0. Thereafter, the fixed IBF was eluted with glycine buffer at pH 2.8. Column eluates and effluents were dialyzed against phosphate-buffered saline and concentrated to the starting volume. Moreover, columns coupled with rabbit IgM or Fab'<sub>2</sub> fragment of IgG were used to obtain control preparations. Rabbit Ig subgroups or Ig fragments were prepared as previously described (13).

*Measurement of IBF activity.* IBF activity was tested by measuring the inhibition of C-induced hemolysis in sheep erythrocytes (SRBC) sensitized with rabbit IgG antibody (reference 13 and footnote 1). The unit referred to in the text is the amount causing 50% inhibition of lysis.

*Spleen Cell Cultures.*  $10^7$  spleen cells from 8- to 12-wk old (C57BL  $\times$  DBA/2)F<sub>1</sub> mice were cultured in 12  $\times$  75 mm Falcon plastic tubes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) in 1

\*This work was supported in part by a grant (no. 74-4-035-1) from the Institut Nationale de la Santé et de la Recherche Médicale.

<sup>1</sup>Guimezanes, A., W. H. Fridman, R. H. Gisler, and F. M. Kourilsky. Manuscript submitted for publication.

ml of Medium RPMI 1640 (Microbiological Associates, Bethesda, Md.) supplemented with 5% fetal calf serum (Rehatuin, Reheis Chemical Co., Chicago, Ill.) and antibiotics (penicillin, streptomycin, 5,000 U each) in a CO<sub>2</sub> incubator (10% CO<sub>2</sub> in air). No nutritive cocktail was added. Cultures were stimulated with  $3 \times 10^6$  SRBC of  $10^{-2}$   $\mu$ g/ml of dinitrophenylated aminoethyl dextran (DNP-AE-DEX) (3). To some cultures graded doses of IBF or control preparations (1–100  $\mu$ l/ml) were added at different times of incubation.

**Plaque Assay.** Direct plaque-forming cells (PFC) were assayed by the local hemolysis technique in liquid medium (17). To detect anti-DNP responses, horse erythrocytes coated with TNP were used as indicator cells (18).

### Results and Discussion

Addition of graded amounts of crude S-ATC or purified IBF to antigen-stimulated spleen cell cultures at initiation of the response significantly suppressed the direct PFC response to SRBC and to the T-independent antigen DNP-AE-DEX (Fig. 1). The inhibitory capacity of both preparations was essentially correlated to their contents of IBF, as determined by their ability to inhibit C-dependent hemolysis, despite the fact that the crude preparations contained 50–60 times more protein than the purified preparations. Thus, purification based on the affinity of IBF for IgG resulted in a clear-cut increase of specific biological activity. However, in no case could the PFC response be totally abolished, suggesting that part of the 19S antibody precursor cells might escape or withstand the effect of IBF. Furthermore, suppression of PFC was not correlated with a decrease in the number of viable cells recovered.

The assumption that IBF was the suppressor is further strengthened by the data shown in Fig. 2. Affinity columns coated with IgM or Fab'2 fragment of IgG, on which IBF was not retained, were also unable to eliminate the soluble inhibitor, whereas effluents from IgG-coated columns, which bind IBF, lost their suppressive properties. If IBF was recycled on IgG-coated columns, both its

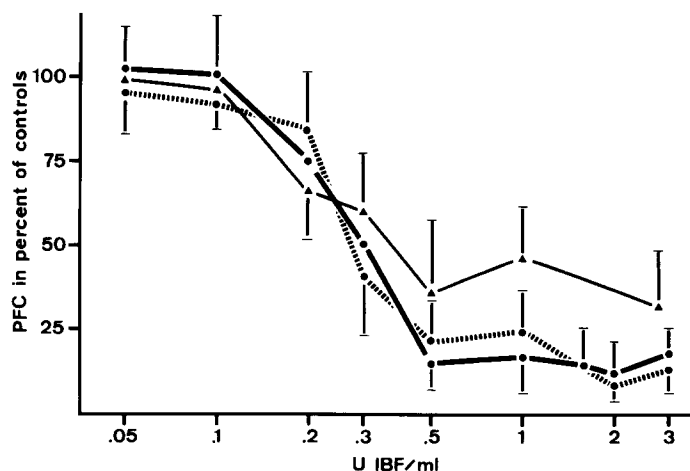


FIG. 1. Effect of IBF (units per milliliter, added together with antigen) on direct PFC formation in spleen cell cultures. Response to SRBC: ●—●, cultures treated with crude supernate of ATC; and ●—●, cultures treated with purified IBF. Response to DNP-AE-DEX: ▲—▲, cultures treated with purified IBF. Each plot represents the mean ( $\pm$  standard deviation) of the PFC counts from five to eight experiments. Plaque responses in control cultures varied between 751–2,613 PFC/ $10^6$  cells against SRBC (day 5) and 279–765 PFC/ $10^6$  cells against DNP-AE-DEX (day 3).

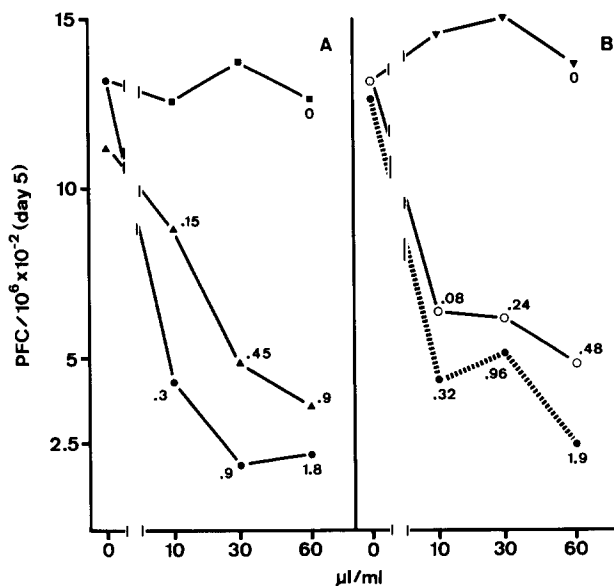


FIG. 2. Effect of different preparations from various types of affinity chromatography column on direct PFC formation against SRBC in spleen cell cultures. All preparations were added at initiation. Numbers at various points on the curves indicate units of IBF. ■ — ■, effluent from IgG-coated column; ▲ — ▲, effluent from Fab'-coated column; ● — ●, effluent from IgM-coated column; ▼ — ▼, effluent from IgG-coated column on which IBF was recycled; ○ — ○, effluent from IgM-coated column on which IBF was recycled; and ● — ●, purified IBF.

ability to block C-dependent hemolysis and its suppressive effect on the PFC response disappeared. Finally, IBF recycled on IgM-coated columns did not bind, and the effluent remained fully inhibitory.

When IBF was added at the start of induction its suppressive effect only became apparent at the peak of the response (R. H. Gisler and W. H. Fridman, unpublished observation). It therefore seems questionable whether it had any effect at all on the induction of the response. From Table I it is clear that neither pretreatment of the cells nor the late addition of IBF (96 h) interfered with the generation of PFC. However, IBF was active when added between initiation and 72 h and displayed maximum inhibitory capacity when added at 72 h. In conclusion, the critical events which finally cause suppression of the PFC response may occur during terminal differentiation of antibody-forming cell precursors. It may be pertinent in this context that suppressor T cells have been found to be most active when added to stimulated spleen cell cultures 48 h after

TABLE I  
Effect of IBF (0.4 U/ml) Added at Different Times on the Response of Spleen Cell Cultures to SRBC

	IBF added before (-), together with (0 h), or after (+) antigen					
	-6 h	0 h	+24 h	+48 h	+72 h	+96 h
PFC, % of controls*	108.5 ± 4.4	51.8 ± 13.6	59.7 ± 10.5	36.0 ± 9.8	13.5 ± 11.3	93.2 ± 6.4

\* Arithmetic means (± standard deviation) of PFC counts (day 5) from seven independent culture experiments. Plaque responses in control cultures varied between 806-3,094 PFC/10<sup>6</sup>.

initiation (19). Similarly, Schimpl and co-workers (5) have associated the opposite phenomenon, namely enhancement of PFC generation by T-cell-replacing factor, with the late phase of terminal differentiation of activated clones. In our case, however, a possible interference with clone expansion cannot, as yet, be fully excluded. Alternatively, the observed suppression of 19S PFC could be due to an IBF-induced switch from the generation of 19S PFC to 7S PFC or to diversion from active antibody synthesis into memory cell pools.

It has already been demonstrated by other workers that soluble inhibitors of humoral antibody synthesis are secreted by ATC (8, 9) or by T-cell lymphomas (10). Since these factors are largely undefined, it is difficult to compare them with IBF. However, in contrast to IBF, the nonspecific inhibitors described by Feldmann (9) and Stocker et al. (10) seem to act only on T-dependent responses. Furthermore, unlike "soluble immune response suppressor" (8), IBF acts best at the late phase of the response.

IBF which is defined by its specificity for the Fc fragment of IgG is produced by ATC (13), some of which are known to bear Fc receptors (13, 20, 21). Recent data suggest that IBF may even be identical with Fc receptors released from ATC.<sup>2</sup> If this proves correct, then according to the foregoing data, the Fc receptor of T cells would appear to have an important part in the regulation of humoral antibody synthesis.

### Summary

Alloantigen-activated mouse T cells secrete a factor which binds to the Fc fragment of IgG and blocks complement (C) activation by IgG (immunoglobulin-binding factor, IBF). IBF was found to suppress the direct plaque-forming cell (PFC) response of mouse spleen cell cultures to sheep erythrocytes and to dinitrophenylated aminoethyl-dextran (T-independent antigen). Purification of IBF by affinity chromatography on IgG-coated Sepharose columns led to an increase of the suppressive capacity of the preparations. In samples from various types of affinity columns (coated with IgG, IgM, or Fab<sub>2</sub> from IgG) the factor responsible for inhibiting the PFC response could not be dissociated from that responsible for the inhibitory activity of IBF on C-dependent hemolysis. No effect was seen when cultures were pretreated for 6 h, or when IBF was added up to 96 h after initiation. On the other hand, IBF was most suppressive when added at 72 h. These data are compatible with the view that IBF is a soluble mediator of suppressor T cells which may interfere with terminal differentiation of antibody-forming cell precursors.

The authors thank Miss V. Bandelier, M. Cristofari, and N. Hell for their expert technical assistance. We are also indebted to Doctors P. Dukor, F. M. Kourilsky, A. Schimpl, and E. Wecker for their helpful comments during the preparation of the manuscript.

*Received for publication 24 April 1975.*

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