Clin. exp. Immunol. (1984) **57**, 155–162.

Modulation of human B cell responses by a monoclonal antibody to an activation antigen 4F2

THERESA L. GERRARD, CYNTHIA H. JURGENSEN & A. S. FAUCI *Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA*

(Accepted for publication 17 February 1984)

SUMMARY

Antigen specific human antibody responses can be modulated *in vitro* by the addition of 4F2 antibody, a monoclonal antibody (MoAb) which recognizes an antigen on activated T cells and B cells. Specific antibody responses induced with the antigen are suppressed by the addition of 4F2. However, specific antibody responses induced with the polyclonal activator, pokeweed mitogen (PWM), are significantly enhanced by the addition of 4F2. Proliferative responses to both antigen and PWM are suppressed by the addition of 4F2. The enhancement of PWM stimulated responses by 4F2 is mediated by T cells. However, in the absence of T cells, 4F2 can directly inhibit antigen specific B cells. Polyclonal Ig production stimulated by PWM was also enhanced by 4F2. Thus, the immunomodulating effects of the 4F2 MoAb are the result of a balance of enhancement and suppression mediated at the T cell and the B cell level, respectively.

Keywords activation antigen 4F2 monoclonal antibodies B cell responses

INTRODUCTION

The development of panels of monoclonal antibodies (MoAbs) that react with various subpopulations of human lymphoid cells has greatly enhanced our ability to identify and precisely delineate the functional capabilities of these subpopulations (Kennett, McKearn & Bechtol, 1980). We still do not fully appreciate the function of the antigens themselves which various MoAbs recognize, yet the relationship of these antigens to the differentiation and functional capabilities of lymphocyte subsets may be critical to our understanding of the role of these subsets in immunoregulation. Study of the effects of MoAbs on *in vitro* human immune responses can provide valuable information in correlating expression of a particular antigen with a specific functional capability.

A MoAb (4F2), which was developed in our laboratory, recognizes a 120,000 dalton glycoprotein on human lymphoid cells which is not an HLA-A,B,C or DR antigen (Haynes *et al.*, 1981). 4F2 binds to an antigen on peripheral blood monocytes as well as activated T lymphocytes. Recently, it has been suggested that the 4F2 antigen may represent an antigen present on activated, but not resting, B lymphocytes (Kehrl & Fauci, 1983). Thus, the expression of the 4F2 antigen on lymphocytes may define a state of activation of T cells as well as B cells. In this regard, investigation of the expression and modulation of the 4F2 antigen could prove useful in our understanding of the regulation of human immune response. The purpose of this study was to examine the effect of the MoAb 4F2 on the *in vitro* induction of T cell-dependent antigen specific human B cell responses.

Correspondence: Dr Theresa L. Gerrard, National Institutes of Health, Room 11B-13, Building 10, Bethesda, Maryland 20205, USA.

MATERIALS AND METHODS

Immunization and cell separations. Normal volunteers were immunized subcutaneously with two 5 mg injections of keyhole limpet haemocyanin (KLH) as previously described (Volkman, Lane & Fauci, 1981; Lane *et al.*, 1981). Heparinized peripheral blood was obtained 2 weeks after the booster immunization, and mononuclear cells were obtained by density gradient centrifugation on Ficoll-Hypaque. The unfractionated mononuclear cells were subsequently washed three times in RPMI 1640 (HEM Research Inc., Rockville, Maryland, USA). The cells were partially depleted of monocytes by adherence to glass beads (4 mm, Kimble, Toledo, Ohio, USA) as previously described (Gerrard & Fauci, 1982). T cells and B cells were first separated by a rosette procedure using S-2-aminoethylisothiouronium bromide hydrobromide (AET) treated sheep red blood cells (SRBC) (Falkoff, Peters & Fauci, 1982). Rosette positive cells were further purified by passage over a nylon wool column. Rosette negative cells were further depleted of any residual T cells by treatment with a monoclonal anti-T cell reagent, anti-Leu 1 (Becton-Dickinson, Sunnyvale, California, USA) and complement as described (Falkoff *et al.*, 1982) or were subjected to a second AET SRBC rosette procedure.

Cultures and assays. Cells were cultured in 96 well flat bottomed dishes (Costar 3596, Cambridge, Massachusetts, USA) in RPMI with 10% fetal calf serum (FCS; Dutchland Laboratories, Denver, Pennsylvania, USA) at a concentration of 3×10^5 cells per well, in a total volume of 220 μl/well. Alternatively, 2×10^5 T cells were cultured with 1×10^5 B cells and monocytes in each well. Cultures were stimulated with various concentrations of KLH or with pokeweed mitogen (PWM; 1:200 stock solution, GIBCO, Grand Island, New York, USA). T cell factors were generated by culturing mononuclear cells from 2 individuals together at 1×10^6 cells/ml with 2 μg/ml phytohaemagglutinin (PHA). Cell free culture supernatants were harvested after 48 h.

After 12 days, culture supernatants were assayed for specific anti-KLH antibody and total Ig synthesis by an enzyme linked immunosorbent assay as previously described (Volkman *et al.*, 1981; Lane *et al.*, 1981). Amounts of specific anti-KLH antibody are expressed as the geometric mean $\overset{\times}{\div}$ s.e. and are determined by relating to standard sera.

MoAbs. The development and characterization of the 4F2 has been described previously (Haynes *et al.*, 1981). For use in cultures, aliquots of ascites fluid containing 4F2 (30 mg/ml protein) that had been stored frozen were used. Dilutions of the 4F2 were added to cultures of unfractionated mononuclear cells. In some experiments, T cells or B cells were treated with 4F2 for 2 h at 37°C then washed before adding to cultures. As a control, another MoAb grown in Ascites fluid, 3A1, was used (Haynes, Eisenbarth & Fauci, 1979). 3A1 is of the same isotype as 4F2.

RESULTS

4F2 suppresses antigen-induced specific antibody responses but enhances PWM-induced specific antibody responses

We have previously demonstrated that mononuclear cells from a donor recently immunized to KLH can be stimulated to produce anti-KLH antibody *in vitro* when cultured with either the immunizing antigen, KLH, or with the polyclonal activator, PWM (Volkman *et al.*, 1981; Lane *et al.*, 1981). PWM stimulates a KLH specific B cell response as well as responses of B cells of varying specificities as part of an overall polyclonal antibody response. On the other hand, when KLH is added to cultures at low concentrations, it stimulates a KLH specific B cell response in the absence of a substantial polyclonal antibody response (Volkman *et al.*, 1981; Lane *et al.*, 1981). We have observed that addition of 4F2 to cultures modulated the production of anti-KLH antibody, but in a divergent manner depending on whether the stimulus was KLH or PWM. Table 1 demonstrates that addition of 4F2 to cultures stimulated with KLH suppressed anti-KLH antibody production. However, the addition of 4F2 to PWM stimulated cultures greatly enhanced the production of anti-KLH antibody. A control MoAb, 3A1, had no effect on the specific anti-KLH antibody response induced with either KLH or PWM. The effect of 4F2 on the proliferative response was also

Table 1. 4F2 modulates lymphocyte responses to antigen and mitogen

Response measured	Antibody added	Stimulus *in vitro* 0	KLH	PWM
Anti-KLH IgM antibody response (units/ml)	0	9 ×÷ 1·2	49 ×÷ 2·2	24 ×÷ 1·3
	4F2	2 ×÷ 1·0	4 ×÷ 1·3	169 ×÷ 1·2
	3A1	8 ×÷ 2·1	58 ×÷ 1·4	40 ×÷ 1·3
Proliferative response (ct/min)	0	320 ± 94	21,707 ± 3,740	14,022 ± 2,728
	4F2	504 ± 157	7,464 ± 1,111	5,781 ± 1,206
	3A1	1,779 ± 380	29,359 ± 5,913	17,132 ± 2,789

Mononuclear cells were cultured with or without 4F2 (1/1000) or 3A1 (1/1,000). For measurement of specific anti-KLH antibody responses, cultures were stimulated with nothing, KLH (50–200 ng/ml) or PWM (1/200), and culture supernatants were assayed for specific antibody after 12 days. Data are expressed as the geometric mean ×÷ s.e. For measurement of proliferation, cultures were stimulated with nothing, KLH (20 μg/ml), or PWM (1/200), and blastogenesis was measured on day 5. Data are expressed as the arithmetic mean ± s.e.

measured (Table 1). Although 3A1 had no effect, the addition of 4F2 inhibited the proliferative response to both KLH and PWM. A titration of the effects of 4F2 are shown in Fig. 1. The suppressive effect of 4F2 on KLH stimulated cultures and the enhancing effect on PWM stimulated cultures were both seen at dilutions of 4F2 of $1/10^5$ of the 4F2 containing ascites fluid.

We have also examined the kinetics of 4F2 modulation of specific anti-KLH responses (Fig. 2). 4F2 added to cultures stimulated with KLH markedly suppressed specific anti-KLH antibody responses even when added up to day 3. However, 4F2 added to antigen stimulated cultures on day 6 or later failed to suppress the production of anti-KLH antibody. In contrast, significant enhancement of specific antibody responses induced with PWM was seen if 4F2 was added on day 0 or day 1. Addition of 4F2 on day 3 caused some enhancement in PWM stimulated responses, and no enhancement was observed if 4F2 was added at day 6 or later. Thus, addition of 4F2 to mononuclear cell cultures could modulate the production of anti-KLH antibody both positively and negatively, depending on whether the stimulus for *in vitro* antibody production was PWM or KLH.

T cells mediate the enhancement of PWM stimulated specific antibody responses by 4F2

In order to determine the cellular level at which 4F2 was able to enhance specific antibody responses stimulated with PWM, we separated mononuclear cells into T cell fractions and B cell fractions. The results of experiments in which T cells or B cells were treated with 4F2, then washed before addition to PWM stimulated autologous untreated B cells or T cells respectively are shown in Table 2. Pre-treatment of T cells with 4F2 caused enhancement of PWM-induced specific antibody whereas pre-treatment of the B cell fraction with 4F2 did not enhance the PWM-induced specific antibody response. Addition of 4F2 to cultures or treatment of the T cells with 4F2 caused a marked enhancement in the PWM-induced production of anti-KLH antibody even with cells from an individual who was a poor responder in the production of anti-KLH antibody (Table 2, Expt 2). Thus, enhancement of specific anti-KLH antibody responses by 4F2 in cultures stimulated with PWM was mediated by T cells.

4F2 can directly suppress B cells

In order to examine the direct effect of 4F2 on B cells, in the absence of T cells, T cell factors were substituted for T cells. Such factors have been shown to stimulate specific anti-KLH antibody synthesis in B cells from a recently KLH immunized donor without stimulation by antigen or mitogen (Peters & Fauci, 1983). The addition of 4F2 to B cells cultured with T cell factors instead of

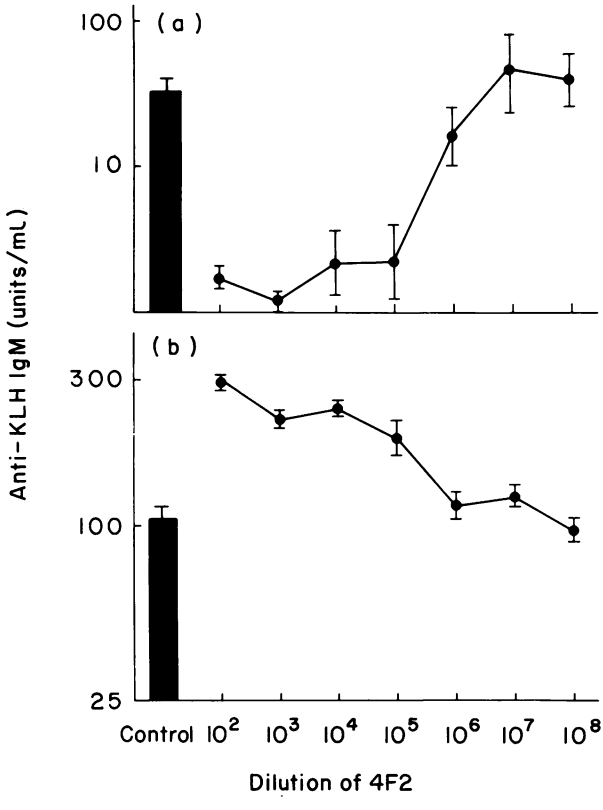

Fig. 1. Unfractionated mononuclear cells were cultured with or without dilutions of 4F2 (4F2 containing ascites fluid = 29 mg/ml protein). Dilutions of 4F2 are reciprocal dilutions of ascites fluid. Cultures were stimulated *in vitro* with either (a) KLH (50–200 ng/ml) or (b) PWM (1/200). Culture supernatants were assayed for specifc anti-KLH antibody on day 12. Data are expressed as geometric means $\overset{\times}{\div}$ s.e.

T cells caused inhibition of anti-KLH specific antibody responses (Table 3). Thus, 4F2 appears to have a direct effect on B cells in inhibiting specific antibody responses.

4F2 enhances PWM stimulated polyclonal Ig

Data in Table 4 demonstrate that the addition of 4F2 to PWM stimulated cultures enhances not only the specific anti-KLH antibody response to the immunizing antigen but also the polyclonal IgM synthesis stimulated by PWM. The percentage increase in antibody synthesis seen with 4F2 caused increased specific or polyclonal antibody responses only in the presence of PWM. In the absence of PWM stimulus, 4F2 did not enhance antibody synthesis. Moreover, any anti-KLH antibody production seen in the absence of any stimulus was suppressed by the addition of 4F2. The percentage increase in PWM-induced anti-KLH IgM production with 4F2 was always somewhat higher than the percentage increase in PWM-induced total IgM production seen with 4F2 addition.

DISCUSSION

We have demonstrated that 4F2 can positively and negatively modulate *in vitro* human B cell responses. It appears that modulation of the 4F2 antigen on lymphocytes can influence cellular interactions that result in a net enhanced or suppressed antibody response. Specific anti-KLH antibody responses induced *in vitro* with the immunizing antigen KLH were suppressed by the addition of 4F2 to culture. In constrast, similar anti-KLH antibody response induced with the

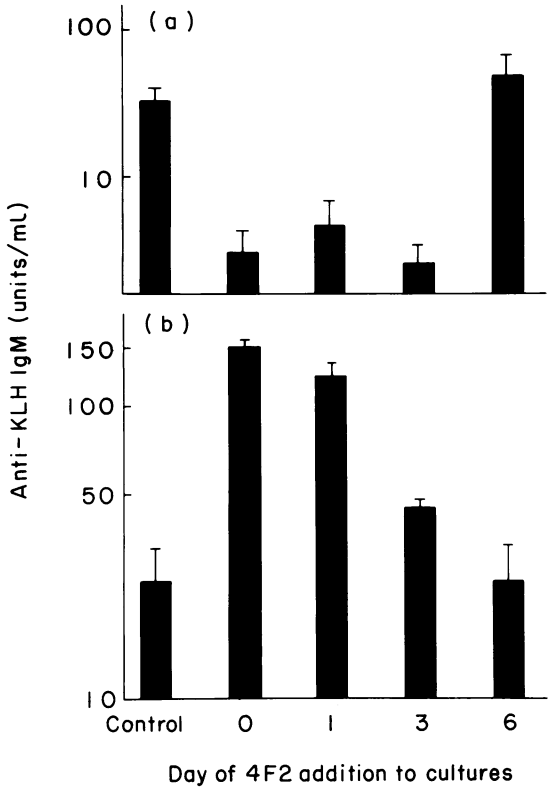

Fig. 2 Unfractionated mononuclear cells were stimulated *in vitro* with either (a) KLH (50–200 ng/ml) or (b) PWM (1/200) from day 0. To some of the cultures, 4F2 (1/200 dilution of ascites fluid) was added on the indicated day. Culture supernatants were measured for anti-KLH antibody on day 12. Data are expressed as geometric means $\overset{\times}{\div}$ s.e.

Table 2. 4F2 acts at the level of the T cell in enhancing the PWM-induced specific anti-KLH response

	Anti-KLH IgM (units/ml) in cultures stimulated with PWM	
	Expt 1	Expt 2
T+B	$57 \overset{\times}{\div} 1{\cdot}5$	$2 \overset{\times}{\div} 1{\cdot}4$
T+B+4F2	$115 \overset{\times}{\div} 1{\cdot}2$	$61 \overset{\times}{\div} 2{\cdot}1$
T treated with 4F2+B	$140 \overset{\times}{\div} 1{\cdot}5$	$87 \overset{\times}{\div} 1{\cdot}7$
T+B treated with 4F2	$58 \overset{\times}{\div} 1{\cdot}6$	$3 \overset{\times}{\div} 3{\cdot}4$

T cells or B cells were treated with 4F2 (1/200 dilution of ascites fluid) for 2 h at 37°C. The treated cells were then washed three times before culturing with autologous untreated B cells or T cells, respectively. Cultures were stimulated with PWM for 12 days. Culture supernatants were measured for anti-KLH IgM synthesis. Data are expressed as geometric means $\overset{\times}{\div}$ s.e.

Table 3. 4F2 can directly suppress B cells

	Anti-KLH IgM (units/ml)		
	Cultures stimulated with		
	0	KLH	PWM
T+B	0	47 ×÷ 1·3	31 ×÷ 1·9
T+B+4F2	0	8 ×÷ 1·5	121 ×÷ 1·1
T factors+B	33 ×÷ 1·0		
T factors+B+4F2	4 ×÷ 1·1		

B cells were cultured with T cells and stimulated with either KLH (10–200 ng/ml) or PWM (1/200). Alternatively, B cells were cultured with T cell factors (10%) without antigen or mitogen stimulation. After 12 days, culture supernates were assayed for anti-KLH IgM. Data are expressed as geometric means ×÷ s.e.

Table 4. 4F2 enhances polyclonal antibody synthesis

		Anti-KLH IgM (units/ml)		Total IgM (μg/ml)	
Expt.	Addition of 4F2	0	PWM	0	PWM
1	–	7 ×÷ 3	74 ×÷ 1	3±0·3	6±0·7
	+	1 ×÷ 1	145 ×÷ 1 (196%)*	2±0·3	10±0·1 (167%)
2	–	7 ×÷ 3	32 ×÷ 1	2±0·3	4±0·7
	+	4 ×÷ 2	217 ×÷ 1 (678%)	0·6±0·1	14±0·9 (350%)
3	–	19 ×÷ 1	108 ×÷ 1	1±0·1	11±0·8
	+	2 ×÷ 2	293 ×÷ 1 (271%)	3±1	21±4 (191%)

Mononuclear cells were stimulated with or without PWM (1/200). 4F2 (1/1,000) was also added to some cultures. After 12 days, culture supernatants were assayed for anti-KLH IgM or total IgM synthesis.

* Percentage increase in antibody synthesis with 4F2.

polyclonal activator PWM were enhanced by the addition of 4F2 to these cultures. Thus, the same KLH specific B cell response was enhanced or suppressed by 4F2 depending on the stimulus for specific anti-KLH antibody production. Proliferative responses to both KLH and PWM were suppressed, although not completely, by the addition of 4F2. Thus, although the proliferative response to PWM was inhibited by 4F2, the PWM-induced specific antibody response was enhanced by 4F2. 4F2 may affect a subpopulation of cells whose proliferation is required for antigen stimulated antibody responses but not for PWM stimulated specific antibody responses. Similar effects have been observed with differential effects of T cell irradiation in antigen- *vs* mitogen-induced specific antibody responses (Lane, Whalen & Fauci, 1983).

The 4F2 antigen has been shown to be present on activated T cells (Haynes *et al.*, 1981) and activated B cells (Kehrl & Fauci, 1983), and it appears that modulation of specific antibody responses by 4F2 is mediated at both the T cell and B cell level. We have shown that enhancement of specific antibody responses induced by PWM occurred when T cells were treated with 4F2. Treatment of the B cell fraction with 4F2 did not enhance the specific response induced with PWM. Thus, 4F2 modulates T cells stimulated with PWM in such a way that there is a net positive effect of T cell help for KLH specific B cells. 4F2 enhances specific antibody responses only with PWM and does not enhance antibody synthesis in the absence of the PWM stimulus. The effect of 4F2 on

PWM stimulated responses was not limited to enhancement of the specifc antibody response against the recent immunogen KLH. Polyclonal IgM responses stimulated by PWM were also enhanced by the addition of 4F2, and this enhancement was only seen with PWM stimulus. Thus, the effect of 4F2 may be to modulate and enhance those cells, most likely T cells, stimulated by PWM.

In contrast to the above findings, we have also demonstrated that 4F2 suppresses anti-KLH antibody responses when these *in vitro* responses are induced with the antigen KLH. Since B cells from a recently immunized donor do not require T cells but need only T cell factors for stimulation of antibody production (Peters & Fauci, 1983), we took the opportunity to examine the direct effect of 4F2 on B cells. In the absence of T cells, 4F2 suppressed specific antibody responses, suggesting that 4F2 has a direct inhibitory effect on B cells. One cannot exclude the possibility that the direct suppressive effect of 4F2 is mediated by monocytes since monocytes express the 4F2 antigen, and monocytes are present in the B cell fraction. However, we have observed that treatment of monocytes with 4F2 has no effect except for that mediated by carry-over via monocyte Fc receptors (Gerrard & Fauci, unpublished observations). Thus, it appears that the suppressive effects of 4F2 occur at the B cell level while the enhancement of PWM stimulated specific responses occurs at the level of the T cell.

There is an apparent discrepancy in the finding that 4F2 suppresses KLH specific B cell responses in KLH stimulated cultures (Table 1), yet 4F2 enhances the KLH specific B cell response in cultures of unfractionated T cells and B cells stimulated with PWM (Fig. 1). One must assume that the 4F2-mediated enhancement of PWM stimulated responses mediated by T cells (Table 2) overcomes the suppressive effect of 4F2 on B cells. The net effect seen with the addition of 4F2 is enhancement of specific antibody production in PWM stimulated cultures. Thus, the apparently disparate results on the effect of 4F2 on specific antibody responses can be explained by the differential effects of 4F2 on B cells as opposed to T cells, and the net balance of effects on enhancement and suppression.

The ability of a MoAb specific for a human lymphocyte antigen to modulate *in vitro* functional responses has been previously demonstrated with the anti-T3 MoAb (Reinherz, Hussey & Schlossman, 1980). Anti-T3 blocked the ability of T cells to provide help for autologous B cells in a PWM driven system and could also suppress the generation of cytotoxic T cells in a mixed lymphocyte reaction. In contrast, we have shown that 4F2 can enhance as well as suppress *in vitro* lymphocyte responses.

In summary, we have shown that a MoAb, 4F2, which defines an activation antigen on human T cells and B cells, can modulate *in vitro* specific antibody responses. It can modulate human T cell and B cell funtion which can result in enhancement or suppression of *in vitro* immune responses depending on the stimulus employed. Such studies may help delineate the precise role of cell surface antigens in cellular interactions and immunoregulation of human immune responses.

We wish to thank Ann London for her editorial assistance.

REFERENCES

FALKOFF, R.M., PETERS, M. & FAUCI, A.S. (1982) T cell enrichment and depletion of human peripheral blood mononuclear cell preparations. Unexpected findings in the study of the functional activities of the separated populations. *J. Immunol. Meth.* **50,** 39.

GERRARD, T.L. & FAUCI, A.S. (1982) Activation and immunoregulation of antigen-specific human B lymphocyte responses: multifaceted role of the monocyte. *J. Immunol.* **128,** 2367.

HAYNES, B.F., EISENBARTH, G.S. & FAUCI, A.S. (1979) Human lymphocyte antigens: production of a monoclonal antibody which defines functional thymus-derived lymphocyte subsets. *Proc. Natl. Acad. Sci. USA.* **76,** 5829.

HAYNES, B.F., HAMLER, M.E., MANN, D.L., EISENBARTH, G.W., SHELHAMER, J., MOSTOWSKI, H.S., THOMAS, C.A., STROMINGER, J.L. & FAUCI, A.S. (1981) Characterization of a monoclonal antibody (4F2) that binds to human monocytes and to a subset of activated lymphocytes. *J. Immunol.* **126,** 1409.

KEHRL, J.H. & FAUCI, A.S. (1983) Identification, purification, and characterization of antigen-activated and antigen-specific human B lymphocytes. *J. exp. Med.* **157,** 1692.

KENNETT, R.H., MCKEARN, T.J. & BECHTOL, K.B. (eds.) (1980) *Monoclonal Antibodies*. Plenum Press, New York.

LANE, H.C., VOLKMAN, D.J., WHALEN, G. & FAUCI, A.S. (1981) *In vitro* antigen-induced antigen-specific antibody production in man. Specific and polyclonal components, kinetics, and cellular requirements. *J. exp. Med.* **154,** 1043.

LANE, H.C., WHALEN, G. & FAUCI, A.S. (1983) Antigen-induced human T cell help: precursor frequency, radiation sensitivity, and allogeneic effects. *J. clin. Invest.* **72,** 636.

PETERS, M. & FAUCI, A.S. (1983) Selective activation of antigen-specific human B cells in recently immunized individuals by nonspecific factors in the absence of antigen. *J. Immunol.* **130,** 678.

REINHERZ, E.L., HUSSEY, R.E. & SCHLOSSMAN, S.F. (1980) A monoconal blocking human T cell function. *Eur. J. Immunol.* **10,** 758.

VOLKMAN, D.J., LANE, H.C. & FAUCI, A.S. (1981) Antigen-induced *in vitro* antibody production in humans: a model for B cell activation and immunoregulation *Proc. Natl. Acad. Sci. USA.* **78,** 2528.