

Lymphocyte Mitogenesis Induced by Monoclonal Antibodies to the T3 Complex. Differential Modulation by Human IgG

CONSTANTINE D. TSOUKAS, JOHN LAMBRIS,* MARTIN LOTZ,
MARY A. VALENTINE, JOHN H. VAUGHAN, AND DENNIS A. CARSON

*Departments of Basic and Clinical Research (BCR4) and *Immunology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037*

Received May 1, 1984; accepted June 18, 1984

Murine monoclonal antibodies OKT3 (IgG₂), 64.1 (IgG₂), and Leu 4 (IgG₁) react with a common membrane antigen on human T cells and induce potent mitogenesis at concentrations of 1 ng/ml, 10 ng/ml, and 100 ng/ml, respectively. Human serum inhibits the mitogenic effect of antibodies OKT3 and 64.1, but not that of Leu 4. The inhibitor in serum has been identified as immunoglobulin G (IgG) as evidenced by the ability of anti-human IgG-Sepharose affinity columns to retain the inhibitory activity. Various immunoglobulin classes and subclasses obtained from human myelomas differ in their ability to inhibit the OKT3-induced activation. The best inhibition is obtained with the IgG subclasses IgG₁ and IgG₃, followed by IgG₂; IgG₄, IgM, and IgA have little if any effect. None of the IgG subclasses inhibit the Leu 4-induced mitogenesis. Indomethacin as well as supernatants containing interleukin 2 (IL-2) can reverse the inhibitory effects of IgG. Prostaglandins (PGE1 and PGE2) inhibit both the OKT3- and Leu 4-induced mitogenesis, thus lacking the selectivity seen with IgG. Since stimulation by the monoclonal antibodies requires the participation of monocytes, an interpretation consistent with the present data is that IgG stimulates monocytes via its Fc portion to release prostaglandins and/or other suppressor factors via an indomethacin-sensitive pathway. The inability of IgG to inhibit Leu 4-induced mitogenesis may therefore relate to an inability of the monocyte subpopulation, which mediates the Leu 4 response, to secrete suppressor factors. These data suggest a potential value of the mitogenic monoclonal antibodies as probes in studying monocyte heterogeneity and T-cell-monocyte interactions. © 1984 Academic Press, Inc.

INTRODUCTION

The development of monoclonal antibodies reactive with human T-lymphocyte surface antigens has aided in the characterization of human T-cell subsets and the identification of molecules crucial in lymphocyte function. The molecular complex identified by monoclonal antibodies OKT3 and Leu 4 is found on all mature T lymphocytes and is associated with many T-cell functions (1-9). One of the interesting properties of antibodies OKT3 and Leu 4 is their ability to trigger resting T cells to proliferate (2, 3). The mitogenic effect of OKT3 requires the intact antibody molecule for optimal response (2, 3), and the presence of monocytes (10). Several investigators have reported heterogeneity in the ability of monocytes to participate in the response to mitogenic monoclonal antibodies (11-13). Although lymphoid cells of all the individuals tested responded well to antibody OKT3 (IgG₂), only 50-60% of the donors responded to antibody Leu 4 (IgG₁).

Human plasma or serum, as well as aggregated IgG, block OKT3-induced mitogenesis (3, 10). In the present investigation, we show that the inhibitory effects of human serum toward OKT3-induced mitogenesis are mediated entirely by IgG. The IgG acts via an indomethacin sensitive pathway. In marked contrast, human serum and purified IgG have no effect on the mitogenic properties of monoclonal antibody Leu 4 which reacts with the same molecular complex on T cells as OKT3.

MATERIALS AND METHODS

Cells and reagents. Peripheral blood mononuclear cells were isolated from normal healthy adults by Ficoll-Hypaque fractionation (14) and were stored in liquid nitrogen until used (15). Serum was also collected and stored at -20°C until needed. All cell cultures were performed in RPMI 1640 medium supplemented with 10% heat-inactivated (56°C for 30 min) fetal bovine serum (FBS),¹ 100 units/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, and 2 mM L-glutamine (all from M. A. Bioproducts, Los Angeles, Calif.). Human myeloma IgG subclasses were kindly provided by Dr. Hans Spiegelberg (Department of Immunology, Scripps Clinic and Research Foundation). These proteins have been previously described (16). Human IgG was prepared by DE-52 chromatography of γ -globulin from human Cohn fraction II, (ICN Pharmaceuticals, Inc., Cleveland, Ohio). Indomethacin, prostaglandin E1 (PGE1), and PGE2 were obtained from Sigma Chemical Company (St. Louis, Mo.). Interleukin 2 (IL-2)-containing culture supernatants were prepared in our laboratory as previously described (17). Phytohemagglutinin was removed by ammonium sulfate precipitation (18) and IL-2 activity was determined as described by Smith and Ruscetti (19).

Mitogenic assay. Peripheral blood mononuclear cells, 2×10^5 per well of flat-bottom trays (Costar, 3596, Cambridge, Mass.), were incubated with an optimal quantity (see Fig. 1) of monoclonal antibody OKT3 (Ortho Diagnostic Systems, Raritan, N.J.), Leu 4 (Beckton-Dickinson, Mountain View, Calif.), or 64.1 (New England Nuclear Corp., Boston, Mass.) in the presence of various concentrations of human serum or IgG. Cultures were incubated in triplicate for 3 days at 37°C in a humidified atmosphere of 5% CO_2 -air. One μCi of [^3H]thymidine was added per well during the last 5 hr of incubation. The cells were collected on glass-fiber filters and washed using a multiharvester system (Titertek Skatron, Flow Laboratories, McLean, Va.). Radioactivity was determined in a liquid scintillation spectrometer.

Fractionation of human serum. Human serum was fractionated into IgG-enriched and IgG-depleted fractions by DE-52 chromatography in 0.01 M phosphate buffer, pH 8.0, followed by 0.3 M NaCl in the same buffer (Whatman Ltd., Maidstone, England). The two fractions were concentrated in Aquacide II-A (Calbiochem, La Jolla, Calif.) and dialyzed against isotonic phosphate-buffered saline (PBS). The IgG-enriched fraction was circulated for 24 hr (4°C) through an affinity column of cyanogen bromide-activated Sepharose 4B (Sigma) that was conjugated either with goat anti-human IgG (Cappel Laboratories, Cochranville, Pa.) or with human IgG that was prepared from Cohn fraction II, as described above. The bound fractions were eluted with 0.05 M glycine, 0.5 M NaCl, pH 3.0. Both bound and nonbound fractions were concentrated, dialyzed in PBS, and tested for ability to inhibit OKT3 and Leu 4 mitogenesis.

¹ Abbreviations used: IL-2, interleukin 2; PG, prostaglandin; FBS, fetal bovine serum.

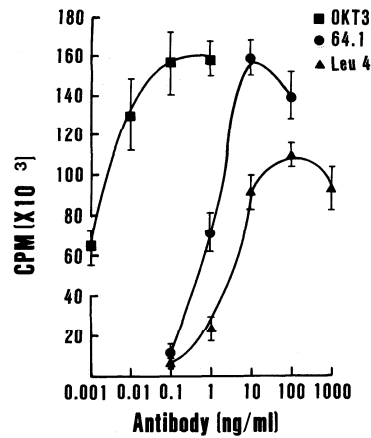


FIG. 1. Mitogenic effects of monoclonal antibodies on human lymphoid cells. Peripheral blood mononuclear cells were incubated as described under Materials and Methods with the indicated concentrations of monoclonal antibodies. Results are expressed as cell-incorporated radioactivity (cpm) at different monoclonal antibody concentrations. The data represent one of two replicate experiments that yielded similar results. Each point represents the average of triplicate determinations (\pm SD).

RESULTS

Effect of Human Serum on the Mitogenic Properties of Monoclonal Antibodies OKT3, Leu 4, and 64.1

Monoclonal antibodies OKT3, Leu 4, and 64.1 displayed mitogenic activity toward human peripheral blood mononuclear cells (Fig. 1). Monoclonal antibody OKT3 was optimally mitogenic at 1 ng/ml, Leu 4 at 100 ng/ml, and 64.1 at 10 ng/ml. All subsequent studies were performed at these concentrations.

Human serum inhibited the mitogenic effect of monoclonal antibodies OKT3 and 64.1 in a dose-dependent manner (Fig. 2). In contrast, monoclonal antibody Leu 4 mitogenesis was not affected at any serum concentration (Fig. 2). Identical results were obtained when cells were preincubated with monoclonal antibodies ($1 \mu\text{g}/2 \times 10^6$ cells), washed and then cultured with serum (data not shown). Inhibition was dependent on the time of addition of serum. Highest inhibition was obtained when serum was added at the initiation of the culture (Fig. 3). Delaying the addition by only 5 hr reduced the inhibition by 50% (Fig. 3).

Identification of the Serum Inhibitor

The serum activity that inhibited OKT3-induced mitogenesis eluted in the low-ionic-strength (IgG-enriched) effluent of a DE-52 ion-exchange column (Table 1). The DE-52 active fraction was analyzed further on an affinity column of goat anti-human IgG coupled to Sepharose 4B (Table 2). The OKT3-inhibitory activity was removed by the anti-IgG column, but not by a control IgG column. Taken together, these results indicated that IgG was the component in human serum that inhibited OKT3- and 64.1-induced lymphocyte mitogenesis. To confirm this result, we tested the effects of human IgG, and IgG subclasses, on the OKT3-induced mitogenesis. Purified human IgG and IgG myeloma proteins of different subclasses inhibited the

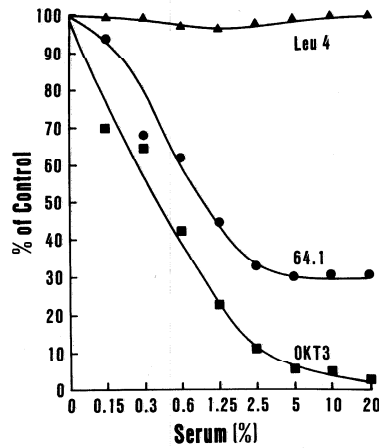


FIG. 2. Inhibition of monoclonal antibody mitogenesis by human serum. Peripheral blood mononuclear cells were incubated as described under Materials and Methods with various amounts of human serum in the presence of an optimal amount of monoclonal antibodies OKT3 (1 ng/ml), 64.1 (10 ng/ml), or Leu 4 (100 ng/ml). Results are expressed as percentages of the radioactivity in control cultures without serum. Control cultures receiving antibody OKT3 incorporated 150,600 cpm; antibody 64.1, 145,750 cpm; and antibody Leu 4, 112,120 cpm. The results represent one of two replicate experiments. Each point is the average of triplicate determinations.

OKT3-induced mitogenesis to various degrees (Fig. 4). The most potent inhibition was produced by the IgG₁ and IgG₃ subclasses, followed by IgG₂ and IgG₄. Additional IgG myelomas tested displayed the same order of subclass inhibitory activity as the one seen in Fig. 4, while IgM and IgA myelomas did not inhibit (not shown). The effects of the myelomas on the mitogenic effects of antibody 64.1 were identical to these on OKT3 (not shown). The purified myeloma proteins, like human serum, displayed little if any effect on the mitogenicity mediated by antibody

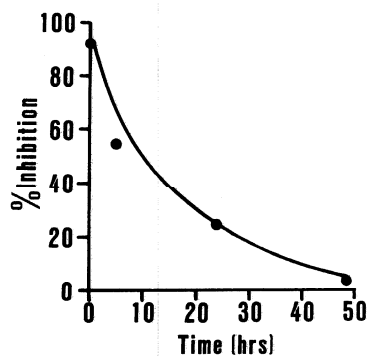


FIG. 3. Timed addition of human serum to OKT3-stimulated cultures. Peripheral blood mononuclear cells were incubated as described under Materials and Methods in the presence of an optimal amount of antibody OKT3 (1 ng/ml). Human serum (2% of total culture volume) was added at culture initiation or various times thereafter. Results are expressed as percentage inhibition, calculated on the radioactivity incorporated by cultures receiving no serum (135,750 cpm). Each point is the average of triplicate determinations. The data are those of one experiment.

TABLE 1
Ion-Exchange Chromatography of Serum Inhibitory Component^a

Fraction	U/mg
Serum	25
IgG enriched	56
IgG depleted	2

^a Human serum was fractionated through DE-52 ion-exchange column as described under Materials and Methods. The IgG-enriched and -depleted fractions were concentrated, dialyzed into PBS, and then tested for inhibition of OKT3 mitogenesis on lymphoid cells. The results are reported as units (U) of activity/mg protein. One unit of activity is the amount causing 50% inhibition of mitogenesis as determined from titration curves. Protein content was determined spectrophotometrically. Results are representative of four replicate experiments.

Leu 4 (Fig. 4). Heat aggregation of the myelomas (65°C for 20–30 min) did not alter the order of their inhibitory ability nor did it result in any inhibition of antibody Leu 4 mitogenesis (not shown).

Effect of Indomethacin, Prostaglandins, and IL-2 Supernatants on Monoclonal Antibody-Induced Mitogenesis

Indomethacin, a prostaglandin synthetase inhibitor (20), reversed the IgG-mediated inhibition of OKT3 mitogenesis (Table 3). PGE₁ and PGE₂ inhibited the mitogenic effects of all three monoclonal antibodies in a dose related fashion (Table 4). Thus, this direct effect of PG did not have the selectivity seen with IgG, which preferentially inhibited antibody OKT3- and 64.1-, but not Leu 4-induced mitogenesis.

IL-2-containing supernatant fluids were also able to reverse the IgG-mediated blockade of mitogenesis (Table 5).

DISCUSSION

Human serum IgG at microgram quantities inhibited the mitogenic effect of monoclonal antibodies OKT3 and 64.1 on human T lymphocytes, but had no effect on the mitogenic effects of monoclonal antibody Leu 4. The order of inhibition by

TABLE 2
Goat Anti-Human IgG-Sepharose 4B Affinity Chromatography of Serum Inhibitor^a

Affinity column	Activity (U/mg)	
	Bound	Nonbound
Anti-IgG	167	<0.1
IgG	<1	150

^a An IgG-enriched fraction prepared by DE-52 ion-exchange chromatography was circulated through an affinity column of activated-Sepharose 4B that was conjugated either with goat anti-human IgG or with human IgG (see Materials and Methods). The OKT3 inhibitory activity was determined as described in Table 1.

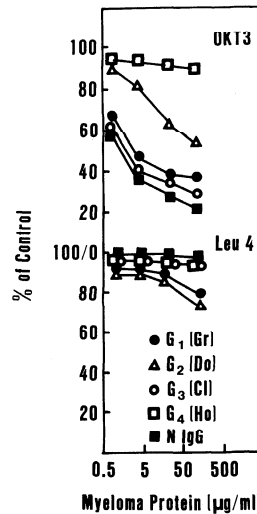


FIG. 4. Effects of human IgG myelomas on antibodies OKT3 and Leu 4 mitogenesis. Peripheral blood mononuclear cells were incubated as described under Materials and Methods with an optimal amount of antibody OKT3 (1 ng/ml) or Leu 4 (100 ng/ml) in the presence of various amounts of normal human IgG (NIgG) or IgG myelomas as indicated. Results are expressed as percentages of radioactivity incorporated by control cultures which received only monoclonal antibodies. Control cultures that received OKT3 incorporated 101,250 cpm and cultures receiving Leu 4, 98,652 cpm. Each point represents the average of triplicate determinations. The data represent those of four replicate experiments.

purified myelomas of various subclasses was $IgG_1 = IgG_3 > IgG_2 > IgG_4$. This pattern is similar to the relative avidity of binding of IgG subclasses to Fc receptors on monocytes (16). The results suggest that the inhibitory effects of IgG toward OKT3 and 64.1 mitogenesis are mediated via the interactions with Fc receptors on monocytes or other accessory cells.

Van Wauwe and Goossens have demonstrated that only aggregated IgG inhibited the OKT3-induced mitogenesis (10). Their data also suggested that the inhibition was mediated via an indomethacin-sensitive pathway. Although, in the experiments shown here, we did not intentionally aggregate IgG, we cannot exclude the presence

TABLE 3
Effect of Indomethacin on the IgG-Mediated Inhibition of Mitogenesis Mediated by the Monoclonal Antibody OKT3^a

Additions	cpm
OKT3	50293 ± 2951
OKT3 + indomethacin	51076 ± 122
OKT3 + IgG	29322 ± 126
OKT3 + IgG + indomethacin	46414 ± 4456

^a Peripheral blood mononuclear cells were incubated in medium containing OKT3 (1 ng/ml), human IgG (10 µg/ml), and indomethacin (1 µg/ml) as indicated. The results show the average of triplicate determinations (±SD) from one of two replicate experiments.

TABLE 4
Effects of Prostaglandins on Monoclonal Antibody-Induced Mitogenesis^a

Prostaglandin	Concentration ($\mu\text{g/ml}$)	cpm		
		OKT3	LEU 4	64.1
PGE1	100	50,351 \pm 5450	56,490 \pm 3743	45,630 \pm 11,544
	10	70,890 \pm 7907	66,554 \pm 2372	57,037 \pm 6,016
	1	94,509 \pm 7297	95,510 \pm 16348	89,993 \pm 9,007
PGE2	100	60,289 \pm 4392	54,188 \pm 4316	67,178 \pm 9,988
	10	86,517 \pm 6785	67,797 \pm 5112	96,330 \pm 4,954
	1	96,323 \pm 5913	100,835 \pm 14167	124,215 \pm 10,395
None	—	103,083 \pm 7116	102,543 \pm 12426	126,751 \pm 23,424

^aPeripheral blood mononuclear cells were incubated as described under Materials and Methods with monoclonal antibodies OKT3 (1 ng/ml), Leu 4 (100 ng/ml), or 64.1 (10 ng/ml) in the presence of PGE₁ or PGE₂. [³H]Thymidine incorporation was measured after 3 days in culture. The results show the average cpm of duplicate cultures (\pm SD) from one of two replicate experiments.

of small complexes during culture. However, in some experiments where IgG was heat aggregated, we could not augment inhibition by IgM, IgA, and IgG₄ myelomas nor could we see any inhibition of mitogenesis mediated by antibody Leu 4. These data then suggest that although aggregation might be necessary for inhibition to occur, it is not sufficient by itself and the IgG class and subclass are important.

Monoclonal antibodies OKT3, Leu 4, and 64.1 all react with a molecular complex on the surfaces of T lymphocytes that contains a predominant 20,000-Da glycoprotein ((21-25) and New England Nuclear Technical Bulletin). The importance of this molecular complex in T-lymphocyte activation is exemplified by the observation that its presence on the cell surface correlates with immune competence, while its absence correlates with unresponsiveness (26). In addition, experiments in our own laboratory have shown that incubation of T cells with antibody OKT3 and an anti-mouse antiserum under conditions that mediate capping and endocytosis of the OKT3-reactive molecule, resulted in reduced ability of the lymphocytes to respond

TABLE 5
Effect of IL-2-Containing Supernatants on the Inhibition of OKT3 Mitogenesis^a

Additions	cpm
OKT3	99,577 \pm 2342
OKT3 + IgG	15,703 \pm 981
OKT3 + IgG + IL-2	78,762 \pm 2161
IL-2	6,559 \pm 132
None	2,200 \pm 564

^aPeripheral blood mononuclear cells were cultured with antibody OKT3 (1 ng/ml), human IgG (30 $\mu\text{g/ml}$), and IL-2 (10 units of activity per ml of a supernatant from PHA-activated lymphoid cells, assayed as previously described (19)). The results show the average of triplicate determinations (\pm SD) from one of two replicate experiments.

to phytohemagglutinin or concanavalin A (M. Valentine, unpublished). The respective determinants recognized on the reactive antigen by the three monoclonal antibodies have not been characterized. There is evidence to suggest that these monoclonal antibodies react with distinct epitopes. Garson *et al.* (27) reported that antibody Leu 4 reacts with Purkinje neurons, while antibody OKT3 does not. Although antibody Leu 4 reacts with 70–90% of human thymocytes (28), OKT3 reacts only with 10% (1). In addition, crossblocking experiments in this laboratory (C. Tsoukas, unpublished observations) have indicated that antibody 64.1 does not interfere with the binding of OKT3 and Leu 4. Monoclonal antibodies OKT3 and 64.1 are of the IgG₂ subclass ((11) and New England Nuclear Technical Bulletin) while antibody Leu 4 is an IgG₁ (11).

The mechanism of T-cell activation by mitogenic monoclonal antibodies is not known precisely. The available data suggest that the antibody binds to its reactive molecule on T cells, followed by binding to monocytes apparently via its Fc portion (2, 3, 10). This scheme is supported by the observations that the Fab (2, 3) and F(ab')₂ (C. Tsoukas unpublished) enzymatic fragments of antibody OKT3 are weakly if at all mitogenic and that depletion of monocytes (10) prevents the mitogenic response of lymphocytes toward the intact antibody. However the mechanism by which monocytes contribute in the response is not clearly understood. One possibility, which is consistent with current themes of T-cell activation, is that monocytes release IL-1, which in turn stimulates the production of IL-2 by T cells (29). IL-2 would then support the T-cell proliferative activity (19). Consistent with this proposed mechanism, IgG inhibited mitogenesis only when added within the first 5 hr after lymphocyte triggering with monoclonal antibodies, and IL-2 abrogated the IgG-inhibitory effect.

Several investigators have recently reported polymorphism in the response of human T lymphocytes to mitogenic murine monoclonal antibodies (11–13). Although the lymphoid cells of all individuals tested were stimulated by mitogenic antibodies of the IgG₂ subclass (e.g., OKT3), only 50–60% of the same donors responded to antibodies of the IgG₁ subclass (e.g., Leu 4). It was shown that nonresponsiveness reflected the inability of the monocytes from the nonresponder donors to interact with the murine IgG₁ antibodies. Tax *et al.* (11) as well as Kaneoka and colleagues (13) observed that monocytes from Leu 4 nonresponder individuals did not display any suppressor or cytotoxic activities. Furthermore, experiments in our laboratory have indicated that addition of indomethacin does not alter the nonresponder status (M. Lotz, unpublished). This finding suggests that lack of response is not due to release of PG or other factors produced via an indomethacin-sensitive pathway. However, addition of lectin-free, IL-2-enriched culture supernatants to antibody Leu 4-nonresponding cultures could render them responsive (C. Tsoukas, unpublished). This observation supports the contention that interleukins are involved in the response to the mitogenic monoclonal antibodies. Taken together the above data suggest the presence of more than one monocyte subset or heterogeneity in monocyte function.

The addition of PGE₁ and PGE₂ to the cultures nonselectively blocked the mitogenic effects of OKT3, Leu 4, and 64.1. In contrast, human IgG only inhibited OKT3 and 64.1 mitogenesis. One interpretation of these results is that prostaglandins are not involved in the IgG-mediated inhibition and that indomethacin reversed the inhibition by affecting a different pathway. Alternatively, it is possible that only

the monocyte subset involved in the response to OKT3 and 64.1 (IgG₂), but not the subset responding to Leu 4 (IgG₁) is capable of releasing prostaglandins upon incubation with human IgG. In view of the previous findings that some donors' monocytes do not respond to antibody Leu 4 (11-13), the above interpretation would mark another level of human monocyte functional heterogeneity.

ACKNOWLEDGEMENTS

The authors thank Theresa Wilcoxson for technical assistance, and Jane Uhle and Frances Kral for typing the manuscript. This is publication number 3398BCR from the Research Institute of Scripps Clinic, La Jolla, California. Funding for this research was provided by a grant from the Lilly Research Laboratories and by NIH Grants GM23200, AM21175, and RR00833.

REFERENCES

1. Kung, P. C., Goldstein, G., Reinherz, E. L., and Schlossman, S. F., *Science (Washington, D.C.)* **206**, 347, 1979.
2. Wauwe, J. P. van, DeMey, J. R., and Goossens, J. G., *J. Immunol.* **124**, 2708, 1980.
3. Chang, T. W., Kung, P. C., Gingras, S. P., and Goldstein, G., *Proc. Natl. Acad. Sci. USA* **78**, 1805, 1981.
4. Platsoucas, C. D., and Good, R. A., *Proc. Natl. Acad. Sci. USA* **78**, 4500, 1981.
5. Biddison, W. E., Shearer, G. M., and Chang, T. W., *J. Immunol.* **127**, 2236, 1981.
6. Tsoukas, C. D., Fox, R. I., Carson, D. A., Fong, S., and Vaughan, J. H., *Cell. Immunol.* **69**, 113, 1982.
7. Landegren, U., Ramstedt, U., Axberg, I., Ullberg, M., Jondal, M., and Wigzell, H., *J. Exp. Med.* **155**, 1579, 1982.
8. Tsoukas, C. D., Carson, D. A., Fong, S., and Vaughan, J. H., *J. Immunol.* **129**, 1421, 1982.
9. Meuer, S. C., Hussey, R. E., Hodgdon, J. C., Hercend, T., Schlossman, S. F., and Reinherz, E. L., *Science (Washington, D.C.)* **218**, 471, 1982.
10. Wauwe, J. P. van, and Goossens, J., *Int. J. Immunopharmacol.* **3**, 203, 1981.
11. Tax, W. J. M., Willems, H. W., Reekers, P. P. M., Capel, P. J. A., and Koene, R. A. P., *Nature (London)* **304**, 445, 1983.
12. Wauwe, J. P. van, and Goossens, J. G., *Cell. Immunol.* **77**, 23, 1983.
13. Kaneoka, H., Perez-Rojas, G., Sasasuki, T., Benike, C. J., and Engleman, E. G., *J. Immunol.* **131**, 158, 1983.
14. Boyum, A., *Scand. J. Clin. Lab. Invest. Suppl.* **97**, 77, 1968.
15. Birkeland, S. A., *J. Immunol. Methods* **35**, 57, 1980.
16. Spiegelberg, H. L., Perlmann, H., and Perlmann, P., *J. Immunol.* **116**, 1464, 1976.
17. Inouye, H., Hank, J. A., Alter, B. J., and Bach, F. H., *Scand. J. Immunol.* **12**, 149, 1980.
18. Acuto, O., Cianfriglia, M., Colombatti, M., Chapuis, B., and Nabholz, M., *J. Immunol. Methods* **53**, 15, 1982.
19. Smith, K. A., and Ruscetti, F. W., *Adv. Immunol.* **31**, 137, 1981.
20. Goodwin, J. S., and Webb, D. R., *Clin. Immunol. Immunopathol* **15**, 106, 1980.
21. Agthoven, A. van, Terhorst, C., Reinherz, F., and Schlossman, S., *Eur. J. Immunol.* **11**, 18, 1981.
22. Borst, J., Prendiville, M. A., and Terhorst, C., *J. Immunol.* **128**, 1560, 1982.
23. Borst, J., Prendiville, M. A., and Terhorst, C., *Eur. J. Immunol.* **13**, 576, 1983.
24. Borst, J., Alexander, S., Elder, J., and Terhorst, C., *J. Biol. Chem.* **258**, 5135, 1983.
25. Bergman, Y., Stewart, S. J., Levy, S., and Levy, R., *J. Immunol.* **131**, 1876, 1983.
26. Lamb, J. R., and Feldman, M., *Nature (London)* **308**, 72, 1984.
27. Garson, J. A., Beverley, P. C. L., Coakham, H. B., and Harper, E. I., *Nature (London)* **298**, 375, 1982.
28. Howard, F. D., Ledbetter, J. A., Wong, J., Bieber, C. P., Stinson, E. B., and Herzenberg, L. A., *J. Immunol.* **126**, 2117, 1981.
29. Britton, S., and Palacios, R., *Immunol. Rev.* **65**, 5, 1982.