ENHANCEMENT OF T LYMPHOCYTE FUNCTIONS BY Fc FRAGMENTS OF IMMUNOGLOBULINS

I. Augmentation of Allogeneic Mixed Lymphocyte Culture Reactions Requires I-A- or I-B-Subregion Differences between Effector and Stimulator Cell Populations*

By EDWARD L. MORGAN,‡ MARILYN L. THOMAN,§ and WILLIAM O. WEIGLE

From the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Papain digestion of mammalian immunoglobulin (Ig) results in the production of an Fc fragment which has the ability to activate thymus-derived (T) and bone marrow-derived (B) cells. Murine B cells are induced to proliferate (1–3) and differentiate to polyclonal antibody-secreting cells (4) in the presence of Fc fragments. The Fc fragment-induced activation of B cells requires accessory macrophages (2–4) and T cells (4). Macrophages function by enzymatically digesting the Fc fragments into mitogenic subfragments which are responsible for inducing B cells to proliferate (3, 5). Fc fragments activate T cells (4, 6, 7) to secrete a soluble T cell-replacing factor [(Fc)TRF][1] (7), which in conjunction with Fc subfragment, induces the proliferating cells to differentiate to antibody-producing cells.

In addition to being a potent polyclonal activator, Fc fragments possess adjuvant properties (8, 9). Specific in vivo and in vitro antibody responses are enhanced by both homologous and heterologous Fc fragments. The adjuvant effect was found to be antigen-dose dependent, with the greatest enhancement occurring with suboptimal concentrations of antigen. T cells are the cell population through which the Fc-adjuvant effect occurs (9). This was deduced from the fact that only thymus-dependent antibody responses could be enhanced. Moreover, when interleukin-2 was substituted for T cells, no enhancement was observed. Because the Fc fragment-mediated enhancement of specific antibody response appears to occur through the helper T cell population, it was of interest to determine whether other T cell responses were affected by Fc fragments. The studies in this report reveal that Fc fragments can potentiate T cell-mediated responses such as antigen-induced proliferation and allogeneic mixed lymphocyte culture (MLC) responses. Enhancement of the MLC response occurs only

* Supported in part by grant AI-07007 from the U. S. Public Health Service, grant IM-42J from the American Cancer Society, and Biomedical Research Support grant RRO-5514. Publication 2316 from the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, Calif.

‡ Recipient of U. S. Public Health Service National Research Service Award AI-05813.

§ Recipient of U. S. Public Health Service National Research Service Award AI-06085.

[1] *Abbreviations used in this paper:* C′, complement; CML, cell-mediated lympholysis; Fc(TRF), Fc fragment-induced soluble T cell-replacing factor; FCS, fetal calf serum; E:S, effector:stimulator cell; MLC, mixed lymphocyte culture; OVA, ovalbumin; PBS, phosphate-buffered saline.

when differences between effector and stimulator populations encompass the I-A and/or I-B subregions.

Materials and Methods

Animals. Male mice of the C57BL/6 and CBA/CaJ strains were obtained from The Jackson Laboratory, Bar Harbor, Maine. Male mice of the A.TH, A.TL, A.AL, B10, B10.Br, B10.A, B10.A(2R), B10.A(4R), and B10.A(5R) were obtained from the Scripps Clinic and Research Foundation breeding colony, La Jolla, Calif. The major histocompatibility (H-2) types of all strains used are shown in Table I.

Preparation of Fc Fragments. A human IgG_1 myeloma protein (Fi) was a gift from Dr. Hans L. Spiegelberg, Scripps Clinic and Research Foundation. The IgG_1 was purified by ammonium sulfate fractionation followed by DEAE-cellulose chromatography with 0.01 M phosphate buffer, pH 8, used as the eluent. Fc fragments were obtained by digestion of IgG_1 with papain (Sigma Chemical Co., St. Louis, Mo.) in the presence of L-cysteine (Sigma Chemical Co.) and EDTA (J. T. Baker Chemical Co., Phillipsburg, N. J.) for 5 h (10). After digestion, the material was chromatographed on Sephadex G-100 to remove any undigested IgG. The Fc and Fab fragments were then separated from each other by DEAE chromatography (11). The Fc preparation was found not to be contaminated with endotoxin as described previously (2).

Enrichment of T Cells. T cells were enriched from whole spleen or lymph node preparations by filtration through nylon wool columns (Fenwall Laboratories, Fairfield, Ill.) (12). Contamination of the nylon wool-filtered cells with B cells was monitored by the mitogenic response to bacterial lipopolysaccharide.

Antisera Treatment. Antisera and complement (C′) treatment was performed essentially as recommended by Shen et al. (13). 50×10^6 cells/ml were incubated with an appropriate amount of antisera at 4°C for 30 min. The cells were washed with phosphate-buffered saline (PBS) containing 5% fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, N. Y.) and resuspended in RPMI-1640 (Flow Laboratories, Inc., Rockville, Md.) containing 5% FCS, 1% sodium azide, and 25% C′ in which they were incubated 30 min at 37°C. The C′, a mixture of rabbit and guinea pig (Pel-Freeze Biologicals Inc., Rogers, Ark.) shown to be noncytotoxic for murine lymphocytes, was a gift from Dr. Sharyn M. Walker, Scripps Clinic and Research Foundation. After C′ treatment, the cells were washed in PBS with 5% FCS and resuspended in complete media. Anti-Lyt-1.2 and anti-Lyt-2.2 were obtained from Dr. F. W. Shen, Memorial Sloan-Kettering Cancer Center, New York. Under the conditions employed, 20–30% of spleen cells treated were recovered after Ly antisera and C′ treatment.

Antigen-induced T Cell Proliferation. To obtain primed T cells, ovalbumin (OVA) (Miles Laboratories, Inc., Elkhart, Ind.) was emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) and 50 μl containing 100 μg antigen was injected subcutaneously at the base of the tail. Inguinal lymph nodes were removed 7–9 days postinjection and used for the in vitro stimulation. The lymph node proliferation assay was done according to the method of Corradin et al. (14). Briefly, the inguinal lymph nodes were dissociated into a single cell suspension in PBS and 5% FCS. 4×10^5 cells in RPMI-1640 supplemented with 2 mM L-

glutamine, 1% BME vitamins (Grand Island Biological Co.), 100 U penicillin, 100 μg streptomycin (Microbiological Associates, Walkersville, Md.), 5×10^{-5} M 2-mercaptoethanol, and 10% FCS were cultured with OVA in 0.3 ml final vol in flat-bottomed microtiter plates (3040 Micro Test II; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) for 5 d at 37°C in an atmosphere of 5% CO_2. The amount of proliferation was assessed by incorporation of 1 μCi of [^{3}H]thymidine (New England Nuclear, Boston, Mass.) during a 24-h pulse.

Induction of Primary MLC. T cell-enriched responder spleen cells (2.5×10^5) were cultured with various numbers of irradiated (2,000 rad) (Gamma Cell 40 Small Animal Irradiator; Atomic Energy of Canada Ltd., Ottawa, Canada) stimulator spleen cells at 37°C in 5% CO_2 in a final vol of 0.3 ml in flat-bottomed microtiter plates (3040 Micro Test II). The culture medium consisted of RPMI-1640 (Flow Laboratories) supplemented as described for the antigen-induced proliferation. Unless otherwise stated, the cultures received 1 μCi [^{3}H]thymidine (New England Nuclear) on day 3 and were harvested for scintillation counting on day 4.

Data Analysis. The enhancement index is a term used to describe the adjuvant properties of Fc fragments and is defined as the following:

$$\text{enhancement index} = \frac{\text{counts per minute (Fc + antigen or stimulator cells)}}{\text{counts per minute (antigen or stimulator cells)}}$$

Each experiment was performed a minimum of three times and the results shown are representative of all the data.

Results

Fc Fragment-mediated Enhancement of the Antigen-induced Proliferative Response. It was previously shown that Fc fragments are capable of potentiating both in vivo and in vitro specific antibody responses (8, 9). To determine if the Fc adjuvant activity could be extended to T cell-mediated responses, the antigen-induced T cell proliferative response was assessed. The results in Fig. 1 indicate that Fc fragments have the capacity to enhance antigen-induced proliferative responses. The maximum enhance-

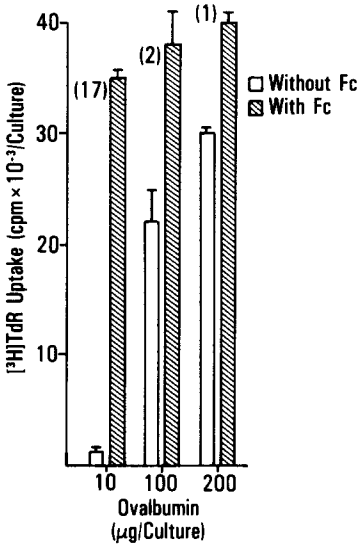

FIG. 1. Nylon wool-purified, lymph node-derived T cells were cultured with increasing amounts of OVA with or without 100 μg/culture Fc. The response was measured on day 5 of culture. Numbers in parentheses indicate enhancement index.

ment (~17-fold) occurred when suboptimal concentrations of OVA were employed. That this enhancement was a result of T cell proliferation is concluded because the lymph node cells used were nylon wool nonadherent and did not respond to bacterial lipopolysaccharide, a B cell mitogen (data not shown). Fc fragments were found not to directly induce T cells to proliferate (Table III) as described previously (1).

It has previously been reported that the lymph node-derived T cell which proliferates to antigen belongs to the Lyt-1^+23^- subclass (15). To confirm this observation in the present model, nylon wool-purified lymph node cells from OVA-primed mice were treated with anti-Lyt-1.2 or Lyt-2.2 before stimulation with antigen. Anti-Lyt-1.2 treatment of the lymph node cells before stimulation totally removed their ability to respond to OVA, whereas the cell population treated with anti-Lyt-2.2 responded as well as or better than the untreated control (Table II). To determine whether the T cell population affected by Fc fragments was the same Lyt phenotype as the population which proliferated in response to antigenic stimulation, the lymph node cells were treated with anti-Lyt-1.2 before culturing. The results reveal that the Fc-induced enhancement (threefold) disappeared when the cells were depleted of the Lyt-1^+23^- subpopulation (Table III). In the reciprocal experiment, prior treatment of the lymph node cells with anti-Lyt-2.2 resulted in no effect on the Fc-mediated enhancement of T cell proliferation (Table IV).

Fc Fragment-mediated Enhancement of the Primary MLC. To further explore the T cell-adjuvant potential of Fc fragments, experimentation was conducted to ascertain whether Fc could enhance the primary allogeneic MLC. The results described in Fig. 2 indicate that the primary MLC between C57BL/6 and CBA/CaJ spleen cells was enhanceable by Fc fragments. Optimal enhancement (16-fold) occurred with a suboptimal effector to stimulator (E:S) ratio. When the cell ratio was such that maximal proliferation was observed, Fc fragments had only a minimal effect. In addition, Fc fragments had no effect on the effector cell population in the absence of allogeneic stimulator cells because the addition of irradiated syngeneic stimulators along with Fc produced no enhancement. To determine if the Fc-mediated potentiation was a result of a shift in the kinetics of the responses, cultures were assayed over a 6-d period. The presence of Fc fragments had no significant effect on the kinetic pattern of the MLC reaction (Fig. 3). Moreover, Fc fragments enhanced the response over the entire culture period.

Major Histocompatibility Complex Difference Requirements to Observe Fc Fragment Enhance-

TABLE II

Ly Phenotype of the Lymph Node T Cells Proliferating to OVA

Treatment*	OVA	[^{3}H]TdR uptake‡
	μg/culture	*cpm/culture*
None	—	2,153 ± 126
None	100	31,872 ± 1,325
Anti-Ly-1.2 + C′	—	4,082 ± 681
Anti-Ly-1.2 + C′	100	7,214 ± 830
Anti-Ly-2.2 + C′	–	2,915 ± 106
Anti-Ly-2.2 + C′	100	69,592 ± 1,873

* Nylon wool-filtered lymph node cells were treated with either anti-Ly-1.2 or anti-Ly-2.2 + C′ before stimulation with OVA.

‡ The response was measured on day 5 of culture.

TABLE III

Inability of Fc Fragments to Enhance OVA-induced Proliferation in Cultures Depleted of Ly-1$^+$ T Cells

Treatment*	OVA	Fc	[^{3}H]TdR uptake‡	Enhancement index§
	μg/culture		*cpm/culture ± SE*	
None	—	—	3,805 ± 33	
None	10	—	6,263 ± 712	
None	—	100	2,844 ± 305	
None	10	100	19,382 ± 932	3
Anti-Ly-1.2 + C′	—	—	4,911 ± 352	
Anti-Ly-1.2 + C′	10	—	5,719 ± 1,422	
Anti-Ly-1.2 + C′	—	100	3,613 ± 238	
Anti-Ly-1.2 + C′	10	100	3,246 ± 1,363	1

* Nylon wool-filtered lymph node cells were treated with nothing or anti-Ly-1.2 + C′ before stimulation with OVA.

‡ The response was measured on day 5 of culture.

§ Enhancement index = $\frac{\text{counts per minute (Fc + OVA)}}{\text{counts per minute (OVA)}}$

TABLE IV

Ability of Fc Fragments to Enhance OVA-induced Proliferation in Cultures Depleted of Ly-23$^+$ T Cells

Treatment*	OVA	Fc	[^{3}H]TdR uptake‡	Enhancement index§
	μg/culture		*cpm/culture ± SE*	
None	—	—	3,580 ± 549	
None	10	—	12,385 ± 779	
None	—	100	2,906 ± 502	
None	10	100	69,607 ± 7,124	6
Anti-Ly-2.2 + C′	—	—	1,809 ± 39	
Anti-Ly-2.2 + C′	10	—	7,109 ± 169	
Anti-Ly-2.2 + C′	—	100	2,231 ± 3,317	
Anti-Ly-2.2 + C′	10	100	77,996 ± 3,630	11

* Nylon wool-filtered lymph node cells were treated with nothing or anti-Ly-2.2 + C′ before stimulation with OVA.

‡ The response was measured on day 5 of culture.

§ Enhancement index = $\frac{\text{counts per minute (Fc + OVA)}}{\text{counts per minute (OVA)}}$

ment of the MLC. It has been reported in the literature that, depending upon the major histocompatibility complex (H-2) difference between effector and stimulator cells, different T cell subsets are stimulated to proliferate. The data expressed in Figs. 2 and 3 were obtained from C57BL/6 (H-2^d) and CBA/CaJ (H-2^k) mice which differed at the H-2 locus as well as at minor histocompatibility loci. To determine if H-2 differences play a role in Fc-induced enhancement, limited H-2 differences were assessed in generation of MLC. The results in Fig. 4 indicate that a difference in the I-A and/or I-B subregion between effector and stimulator cells is essential to obtain an enhanced MLC. This was deduced from the fact that only in experiments where I-A and/or I-B differences were present (groups I, IV, V, VI, and VII) did Fc fragments enhance the primary MLC. The I-region haplotype did not appear to make

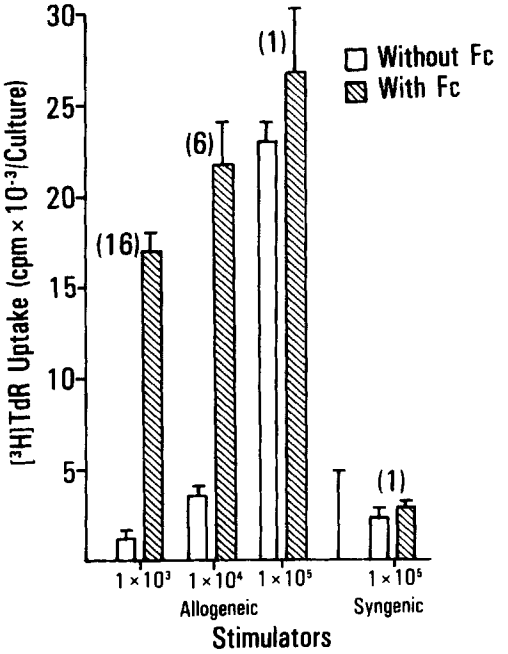

FIG. 2. Nylon wool-purified C57BL/6 splenic T cells were cultured with increasing numbers of irradiated CBA/CaJ spleen cells with or without 100 μg/culture Fc. The response was measured on day 4 of culture.

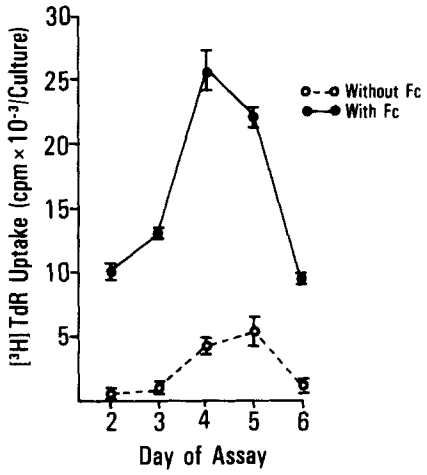

FIG. 3. Allogeneic MLC response between C57BL/6 (responder) and CBA/CaJ (stimulator cell populations). Suboptimal numbers (1×10^4/culture) of stimulator cells, with or without 100 μg/culture Fc, were added to 2.5×10^5 responder cells and the response measured on day 4 of culture.

a difference because the A.TH anti-A.TL MLC (group IV) recognized I^k and the B10.A anti-B10.A(5R) (group V), B10.A anti-B10 (group VI), and B10.Br anti-B10.A(4R) (group VII) all recognized I-A^b and/or I-B^b. The Fc-mediated enhancement of the MLC was not a result of the inability of the other groups to produce an MLC because in all cases, optimal E:S ratios produced a proliferative response

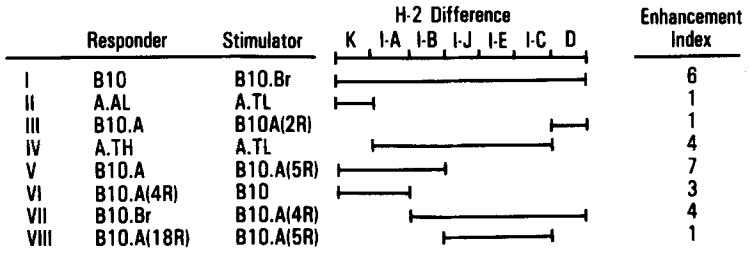

FIG. 4. Allogeneic MLC responses between mouse strains with limited H-2 differences. Suboptimal numbers (1×10^4) of stimulator cells were employed. When optimal E:S ratios were used all strain combinations produced an MLC response ≥20,000 cpm. The responses were measured on day 4 of culture.

TABLE V

Ly Phenotype of the T Cell Proliferating in Response to MHC I-Region Differences

A.TH* responder	A.TL‡ stimulator	[^{3}H]TdR uptake§
		cpm/culture ± SE
No treatment	−	1,426 ± 370
No treatment	+	28,331 ± 1,130
Anti-Ly-1.2 + C′	−	1,324 ± 106
Anti-Ly-1.2 + C′	+	2,724 ± 1,000
Anti-Ly-2.2 + C′	−	1,019 ± 484
Anti-Ly-2.2 + C′	+	24,759 ± 1,190

* Responder cells consisted of 2.5×10^5 nylon wool-nonadherent spleen cells.
‡ Stimulator cells consisted of 2.5×10^5 spleen cells which received 2,000 rad of irradiation before culture.
§ The response was measured on day 4 of culture.

>20,000 cpm (data not shown). A difference in either the K or D region did not appear to be important because in neither case (H-2K^s [group II]; H-2D^b [group III]) was an enhanced response observed.

Because enhancement of the primary MLC occurred only when I-A and/or I-B differences were involved, it became important to determine the Lyt phenotype of the proliferating cell population. Spleen cells from A.TH mice were treated with anti-Lyt-1.2 or Lyt-2.2 before stimulation with A.TL spleen cells. Treatment of the spleen cells with anti-Lyt-1.2 resulted in a population incapable of proliferating in a primary MLC (Table V). In contrast, anti-Lyt 2.2 treatment had no effect on the primary MLC. These results indirectly indicate that the T cell subpopulation acted upon by Fc fragments bears the Lyt-1$^+$23$^-$ phenotype. To directly determine this possibility effector cells were depleted of Lyt-1$^+$23$^-$ cells before mixing with stimulator cells and Fc fragments. The results shown in Fig. 5 demonstrate that the depletion of Lyt-1$^+$23$^-$-bearing T cells removes the ability of Fc fragments to enhance the I-region difference MLC.

Because the D-region difference MLC was not enhanced by Fc fragments, it was deemed important to ascertain the Lyt phenotype of the effector cell. Spleen cells from B10.A mice were treated with anti-Lyt-1.2 or Lyt-2.2 before stimulation with B10.A(2R) spleen cells. The results in Table VI show that treatment of the effector population with either anti-Lyt-1.2 or anti-Lyt-2.2 reduced the proliferation to

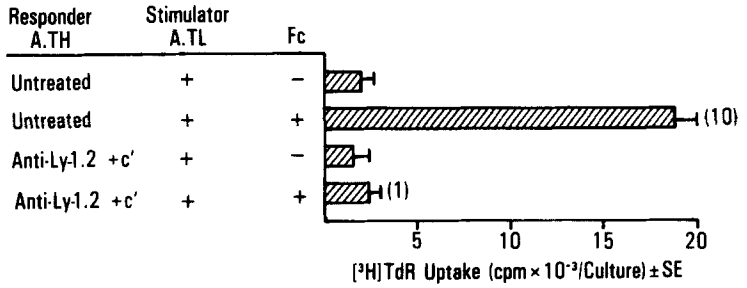

FIG. 5. Allogeneic MLC response between I-region-incompataible strains. The responder (A.TH) population was treated with anti-Lyt-1.2 + C′ before stimulation with suboptimal numbers (1×10^4/culture) of stimulator (A.TL) cells and Fc. The response was measured on day 4 of culture.

TABLE VI

Ly Phenotype of the T Cell Proliferating in Response to MHC D-Region Differences

B10.A* responder	B10.A(2R)‡ stimulator	[³H]TdR uptake§
		cpm/culture ± SE
No treatment	−	1,493 ± 294
No treatment	+	23,040 ± 3,000
Anti-Ly-1.2 + C′	−	1,514 ± 146
Anti-Ly-1.2 + C′	+	1,650 ± 260
Anti-Ly-2.2 + C′	−	1,324 ± 281
Anti-Ly-2.2 + C′	+	1,611 ± 393

* Responder cells consisted of 2.5×10^5 nylon wool-nonadherent spleen cells.

‡ Stimulator cells consisted of 2.5×10^5 spleen cells which received 2,000 rad of irradiation before culture.

§ The response was measured on day 4 of culture.

background levels. Moreover, when mixtures of Lyt-1^+23^- and Lyt-1^-23^+ cells were used as effectors, no significant proliferation was observed (data not shown). These results are interpreted to mean that the cell population stimulated to proliferate in D-region MLC bears the Lyt-123^+ phenotype.

Discussion

The Fc portion of mammalian Ig possesses the ability to enhance, both in vivo and in vitro, specific antibody responses (8, 9). Moreover, this adjuvant effect has been found to occur through the action of Fc fragments on the helper T cell population (9). Evidence is presented in this report showing that T cell-mediated responses, antigen-induced proliferation, and MLC can be enhanced by the presence of Fc fragments. Although Fc fragments do not induce T cells to proliferate (1), they do activate T cells to release lymphokine(s). Thoman et al. (7) recently observed that in the presence of Fc fragments, T cells are stimulated to release a soluble factor termed (Fc)TRF. This factor has been found to replace the need for a T cell signal in Fc fragment-induced polyclonal antibody production.

The T cells acted upon by Fc fragments in the enhancement of both the antigen-induced proliferative and MLC responses possess the phenotypic markers of helper cells. The antigen-primed lymph node T cells which proliferate upon in vitro

stimulation with antigen have also been shown to function as helper cells for antibody production as measured in a secondary hapten-carrier system (15). Moreover, these primed lymph node cells bear the Lyt-1^+23^- phenotype (15). This observation has been confirmed in this report as anti-Lyt-1.2, but not anti-Lyt-2.2, abrogated the ability of OVA-primed T cells to proliferate in response to antigen. In addition, this Lyt-1^+23^- population was the cell population susceptible to the enhancing properties of Fc fragments. The antigen-induced proliferative response of Lyt-1^+23^- cells was enhanced approximately threefold compared with the controls that received antigen alone.

In addition to being able to enhance antigen-induced proliferative responses, Fc fragments potentiated primary in vitro MLC between H-2-disparate strains of mice. The primary MLC could be enhanced by as much as 20-fold if a suboptimal E:S ratio was employed. As previously described for enhancement of antibody responses (8, 9) and antigen-induced proliferation, the optimal conditions for enhancing the MLC require suboptimal numbers of stimulatory cells. The maximal enhancement occurred with limited numbers of stimulator cells.

Of special importance is that Fc fragment-mediated enhancement of MLC is restricted to those responses where the allogenic differences between effector and target cells encompass the I-A and/or I-B subregions. By employing B10 congenic mice, it was observed that differences in I-A and/or I-B subregions were mandatory for obtaining an enhanced MLC. The strain combinations used to map the I-region differences necessary for enhancement contained H-2K-region differences as well. That the H-2K-region differences played no role in the Fc-mediated enhancement was indicated by the fact that I-region differences (A.TH anti-A.TL) produced an enhanceable MLC (fourfold). These data also suggest that a difference in the newly described I-N subregion between H-2K and I-A (16) is not responsible for the enhanced MLC, because H-2K-region-incompatible strains did not lead to an increased proliferative response. Furthermore, the nature of the I-A- and/or I-B region antigenic determinant does not appear to be important because Fc fragments were capable of enhancing MLC where $I\text{-}A^k$,$I\text{-}B^k$ or $I\text{-}A^b$,$I\text{-}B^b$ were recognized as the stimulating determinants. The Lyt phenotype of the T cells responding to I-A- and/or I-B-region differences was found to be Lyt-1^+23^- whereas the nonenhanceable H-2D-region MLC was mediated by Lyt-123^+ cells. The Lyt phenotype of the H-2D-region MLC effector cells was deduced from the experiments where both anti-Lyt-1.2 and anti-Lyt-2.2 treatment reduced the anti-H-2D MLC to background levels.

The studies of Cantor and Boyse (17, 18) indicate that the differentiation of cytotoxic T cells in vitro is associated with the collaboration of an amplifier or helper population with the cytotoxic T cell precursor. The amplifier T cell population appears to be expressed as the proliferating cell in the primary MLC reaction. The recognition of I-region determinants by T cells has been associated with the production of helper cells for the primary cell-mediated lympholysis (CML) response by T cells to H-2K- or D-region determinants (17–21). In addition, the Lyt phenotype of helper cells in the CML response between H-2-disparate strains is an Lyt-1^+23^- T cell (17, 18, 22, 23). Taken together these results are interpreted to mean that Lyt-1^-23^+ cytotoxic T cell precursors (17, 18) recognized H-2K and/or D antigens and in conjunction with Lyt-1^+23^- cells, which recognize I region determinants, produce the CML response. Exceptions to this general model exist when limited H-2 differences

(24–26), syngeneic tumors (27, 28), virus-modified (29), or haptenated (30) syngeneic cells are employed. In all these cases it appears that an Lyt-123$^+$ amplifier or helper cell is required for efficient expression of cytotoxicity. The work described in this report employing limited H-2 differences in MLC reactions confirms that of others (24–26), in that the proliferating cell population generated against H-2D-region differences contains Lyt-123$^+$ cells. The reason for the ability of Fc fragments to potentiate MLC against I-A- and/or I-B-region, but not H-2K- or D-region differences is presently unknown. Because it has been proposed that Lyt-123$^+$ cells are precursors of Lyt-1$^+$23$^-$ and Lyt-1$^-$23$^+$ cells, the possibility exists that these Lyt-123$^+$ cells are an immature population and are unable to respond to the Fc signal. This possibility seems unlikely in light of the work by Swain et al. (31, 32) where they describe the existence of long-lived, mature Lyt-123$^+$ helper cells. These authors (31, 32) found that helper activity for the primary in vitro response to sheep erythrocytes could be induced by recognition of foreign H-2 antigens. Helper activity generated with whole haplotype or I-region differences was derived from Lyt-1$^+$23$^-$ cells, whereas H-2K- or D-region differences produced Lyt-123$^+$ helper cells.

The foregoing results indicate that only certain subpopulations of helper T cells (i.e., Lyt-1$^+$23$^-$) are susceptible to the enhancing properties of Fc fragments. Similar observations have recently been found to hold true for CML responses. Enhanced CML occurred where whole haplotype but not H-2K- or D-region, differences were employed (E. L. Morgan and W. O. Weigle, manuscript in preparation). The nature of the signals provided by the Fc fragments and the role of the Lyt antigens, if any, in the acceptance of a signal by the T cells are unknown and are currently under investigation.

Summary

Fc fragments derived from human Ig were found to be capable of enhancing T cell-mediated, antigen-induced proliferative and mixed lymphocyte culture responses. Maximum enhancement occurred when suboptimal amounts of antigen or suboptimal numbers of stimulator cells were employed. Augmentation of the allogeneic mixed lymphocyte culture reaction requires an I-A and/or I-B subregion difference between effector and stimulator cell populations. Although a significant proliferative response was observed with K- or D-region differences, Fc fragments were unable to enhance the response. The T cell population acted upon by Fc fragments in the potentiation of these responses bears the Lyt-1$^+$23$^-$ phenotype.

We thank Nancy Kantor for excellent technical assistance and Janet Kuhns for secretarial expertise.

Received for publication 2 December 1980.

References

1. Berman, M. A., and W. O. Weigle. 1977. B-lymphocyte activation by the Fc region of IgG. *J. Exp. Med.* **146:**241.
2. Morgan, E. L., and W. O. Weigle. 1979. The requirement for adherent cells in the Fc fragment-induced proliferative response of murine spleen cells. *J. Exp. Med.* **150:**256.
3. Morgan, E. L., and W. O. Weigle. 1980. Regulation of Fc fragment-induced murine spleen cell proliferation. *J. Exp. Med.* **151:**1.

4. Morgan, E. L., and W. O. Weigle. 1980. Polyclonal activation of murine B-lymphocytes by Fc fragments. I. The requirement for two signals in the generation of the polyclonal antibody response induced by Fc fragments. *J. Immunol.* **124:**1330.
5. Morgan, E. L., and W. O. Weigle. Regulation of B-lymphocyte activation by the Fc portion of immunoglobulin. *J. Supramol. Struct.* In press.
6. Thoman, M. L., E. L. Morgan, and W. O. Weigle. 1980. Polyclonal activation of murine B lymphocytes by Fc fragments. II. Replacement of T cells by a soluble helper T cell-replacing factor (TRF). *J. Immunol.* **125:**1630.
7. Thoman, M. L., E. L. Morgan, and W. O. Weigle. 1981. Fc fragment activation of T lymphocytes. I. Fc Fragments trigger Lyt 1^+23^- T lymphocytes to release a helper T cell replacing activity. *J. Immunol.* **123:**632.
8. Morgan, E. L., S. M. Walker, M. L. Thoman, and W. O. Weigle. 1980. Regulation of the immune response. I. The potentiation of in vivo and in vitro immune responses by Fc fragments. *J. Exp. Med.* **152:**113.
9. Morgan, E. L., M. L. Thoman, S. M. Walker, and W. O. Weigle. 1980. Regulation of the immune response. II. Characterization of the cell population(s) involved in the Fc fragment-induced adjuvant effect. *J. Immunol.* **125:**1275.
10. Porter, R. R. 1959. The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain. *Biochem. J.* **43:**119.
11. Spiegelberg, H. L. 1976. Principle of methods. *In* Textbook of Immunopathology. P. A. Miescher and H. J. Mueller, editors. Grune & Stratton, Inc., New York. 101.
12. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3:**645.
13. Shen, F.-W., E. A. Boyse, and H. Cantor. 1975. Preparation and use of Ly antisera. *Immunogenetics.* **2:**591.
14. Corradin, G., H. M. Etlinger, and J. M. Chiller. 1977. Lymphocyte specificity to protein antigens. I. Characterization of the antigen-induced *in vitro* T cell-dependent proliferative response with lymph node cells from primed mice. *J. Immunol.* **119:**1048.
15. Schrier, R. D., B. J. Skidmore, J. T. Kurnick, S. N. Goldstine, and J. M. Chiller. 1979. Propagation of antigen-specific T cell helper function *in vitro*. *J. Immunol.* **123:**2525.
16. Hayes, C. E., and F. H. Bach. 1980. *I-N.* A newly described *H-2 I* subregion between *K* and *I-A*. *J. Exp. Med.* **151:**481.
17. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. *J. Exp. Med.* **141:**1376.
18. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Ly^+ cells in the generation of killer activity. *J. Exp. Med.* **141:**1390.
19. Wagner, H., D. Götze, L. Ptschelinzew, and M. Röllinghoff. 1975. Induction of cytotoxic T lymphocytes against *I*-region-coded determinants: *in vitro* evidence for a third histocompatibility locus in the mouse. *J. Exp. Med.* **142:**1477.
20. Alter, B. J., and F. H. Bach. 1974. Role of H-2 lymphocyte-defined and serologically-defined components in the generation of cytotoxic lymphocytes. *J. Exp. Med.* **140:**1410.
21. Bach, F. H., M. L. Bach, and P. M. Sondel. 1976. Differential function of major histocompatibility complex antigens in T-lymphocyte activation. *Nature (Lond.).* **259:**273.
22. Wagner, H., and M. Röllinghoff. 1978. T-T-cell interactions during in vitro cytotoxic allograft responses. I. Soluble products from activated $Ly1^+$ T cells trigger autonomously antigen-primed $Ly23^+$ T cells to cell proliferation and cytolytic activity. *J. Exp. Med.* **148:** 1523.
23. Okada, M., G. R. Klimpel, R. C. Kuppers, and C. S. Henney. 1979. The differentiation of

cytotoxic T cells *in vitro*. I. Amplifying factor(s) in the primary response is Lyt 1^+ cell dependent. *J. Immunol.* **122:**2527.

24. Wettstein, P. J., D. W. Bailey, L. E. Mobraaten, J. Klein, and J. A. Frelinger. 1978. T-lymphocyte response to *H-2* mutants. I. Proliferation is dependent on Ly 1^+2^+ cells. *J. Exp. Med.* **147:**1395.
25. Okada, M., and C. S. Henney. 1980. The differentiation of cytotoxic T cells *in vitro*. II. Amplifying factor(s) produced in primary mixed lymphocyte cultures against K/O stimuli require the presence of Lyt 2^+ cells but not Lyt 1^+ cells. *J. Immunol.* **125:**300.
26. Bach, F. H., and B. J. Alter. 1978. Alternative pathways of T lymphocyte activation. *J. Exp. Med.* **148:**829.
27. Shiku, H., T. Takahasi, M. A. Bean, L. J. Old, and H. F. Oettgen. 1976. Ly phenotype of cytotoxic T cells for syngeneic tumor. *J. Exp. Med.* **144:**1116.
28. Stutman, O., F. W. Shen, and E. A. Boyse. 1977. Ly phenotype of T cells cytotoxic for syngeneic mouse mammary tumors: evidence for T cell interactions. *Proc. Natl. Acad. Sci. U. S. A.* **74:**5667.
29. Pang, T., I. F. C. McKenzie, and R. V. Blanden. 1976. Cooperation between mouse T cell subpopulations in the cell-mediated response to a natural pox virus. *Cell. Immunol.* **26:**567.
30. Cantor, H., and E. A. Boyse. 1976. Regulation of cellular and humoral responses by T cell subclasses. *Cold Spring Harbor Symp. Quant. Biol.* **41:**23.
31. Swain, S. L., and P. R. Panfili. 1979. Helper cells activated by allogeneic H-2K or H-2D differences have a Ly phenotype distinct from those responsive to I differences. *J. Immunol.* **122:**383.
32. Swain, S. L., A. Bakke, M. English, and R. W. Dutton. 1979. Ly phenotypes and MHC recognition: the allohelper that recognizes K or D is a mature Ly 123 cell. *J. Immunol.* **123:** 2716.