MURINE PEYER'S PATCH T CELL CLONES

Characterization of Antigen-specific Helper T cells for Immunoglobulin A Responses*

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The immunoglobulin A (IgA) response is thymic dependent (TD),[1] because athymic, nude mice exhibit depressed serum IgA levels (1–3) and neonatally thymectomized rabbits do not undergo IgA responses (4). Furthermore, human T cell dysfunctions contribute to IgA deficiency in some individuals (5–7). However, it has been difficult to directly study T cell regulation of IgA responses to specific antigens. Part of this difficulty has been because of nonuniform methods for isolation of T cells from inductive sites and to low frequencies of antigen-specific T helper (T_h) cells in the T lymphocyte population.

A major source of precursor cells for IgA responses is the gut-associated lymphoreticular tissue (GALT), e.g., Peyer's patches (PP), which contain antigen-sensitive T and B cells (8, 9) and accessory cells (MØ) (9–11). Oral immunization of mice with TD antigen sensitizes both T cells (12, 13) and precursor IgA B cells (14) in GALT. These sensitized cells leave the PP via efferent lymphatics and migrate to distant mucosal sites, where final differentiation of IgA precursor B cells leads to IgA expression. Elson and co-workers (15) reported that murine PP T cells, stimulated with concanavalin A (Con A), suppressed IgM and IgG and helped IgA isotype expression in LPS-driven B cell cultures, whereas Con A-treated splenic T cells suppressed all three isotypes. Our previous studies (13) have shown that gastric intubation of sheep erythrocytes (SRBC) induces significant T_h cell activity in the murine PP. Furthermore, in vitro immunization with SRBC of PP cells from orally

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[1] *Abbreviations used in this paper:* BP, Bordetella pertussis vaccine; C, complement; CFA, complete Freund's adjuvant; Con A, concanavalin A; DNP, dinitrophenyl; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; GALT, gut-associated lymphoreticular tissue; HRBC, horse erythrocytes; IL-2, interleukin 2; KLH, keyhole limpet hemocyanin; MEM, minimum essential medium; MHC, major histocompatibility complex; MØ, accessory cells; PP, Peyer's patches; TCGF, T cell growth factor; TD, thymic dependent; T_h, T helper; T_s, T suppressor; TNP, trinitrophenyl; TRITC, tetramethyl rhodamine isothiocyanate; SRBC, sheep erythrocytes.

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primed mice leads principally to IgA responses (11). Richman and colleagues (16) showed that enteric administration of a single large dose of ovalbumin to mice induced T_h cells for IgA responses within 1 d, and T suppressor (T_s) cells, which diminished IgG responses when PP T cells were transferred to syngeneic, immunized hosts. The concomitant induction of antigen-specific T_h and T_s cells in GALT provides a cellular basis for the important observation that oral administration of antigen induces both systemic unresponsiveness (oral tolerance) and salivary IgA responses (17). All of these studies have provided strong suggestive evidence for the existence of T_h cells for IgA responses.

The availability of methods for continuous proliferation of antigen-specific T lymphocyte clones in culture has been a major recent advance in studies directed toward a molecular understanding of T cell function. Watson and colleagues (18-20) described methods for induction and continuous culture of antigen-specific T_h cell clones. The frequency of T_h cells increased with time in culture, and clones could be maintained with T cell growth factor (TCGF) for indefinite periods (19).

In the present study, we adapted the method of Watson (19) to directly isolate and grow T cells from murine PP. Single T cell clones have been established that are antigen specific and dependent upon TCGF for continuous growth. These clones have been maintained for long periods in culture (7 mo). A number of clones exhibit helper activity for IgA responses, and a complete description of these clones is presented.

Materials and Methods

Mice. C3H/HeJ and C57BL/10Sn (original breeders from The Jackson Laboratory, Bar Harbor, ME) and C3H/HeN and BALB/c +/+ and nude (original breeders obtained from the National Institutes of Health, Bethesda, MD) mice were bred and maintained in The Core Facility for Immunocompromised Mice, The Comprehensive Cancer Center at the University of Alabama in Birmingham. All mice used in these studies were 8-12 wk of age.

Preparation of Murine TCGF or Interleukin 2 (IL-2). Single spleen cell suspensions (13) from C3H/HeN mice were cultured in RPMI 1640 medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) supplemented with 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), gentamycin (50 μg/ml), 5×10^{-5} M 2-mercaptoethanol (incomplete RPMI 1640 medium), and 1% fetal calf serum (FCS). Con A (Miles Laboratories, Inc., Elkhart, IN) was added to cultures at a final concentration of 2 μg/ml. Cultures were incubated in a humidified chamber containing an atmosphere of 7% O_2, 10% CO_2, and 83% N_2 at 37°C for 18 h. Cultures were harvested by centrifugation (1,200 rpm, 10 min), and the supernatants were collected and passed through Sephadex G-10 columns (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, NJ) to remove Con A. Residual Con A was removed from the culture supernatant by precipitation with 0.1 M α-methyl-*D*-mannoside, followed by addition of ammonium sulfate to a final concentration of 40% (19). After incubation at 4°C for 12 h, the precipitate was removed by centrifugation, and the supernatant was brought to 80% saturation with ammonium sulfate (19) and incubated an additional 12 h at 4°C with gentle stirring. The precipitate was collected by centrifugation (10,000 *g*, 30 min), dissolved in buffer (0.001 M phosphate and 0.05 M ammonium bicarbonate), and dialyzed extensively against this buffer and finally against RPMI 1640 medium. In some experiments, IL-2 was further purified by fractionation on a calibrated Sephadex G-75 column, and fractions of ~30,000-40,000 mol wt were collected and concentrated. IL-2 activity was assessed by replacement of T cell help in BALB/c nude spleen cell cultures immunized with sheep erythrocytes (SRBC), as previously described (19). In our experiments, we arbitrarily assigned 1 U of IL-2 activity as the amount required to support immune responses of >200 IgM anti-SRBC PFC/culture in nude spleen microcultures (5×10^5 cells/0.2 ml). Approximately 1-2 U of IL-2/ml was present in unfractionated supernatant, and at least 80% recovery was obtained after all subsequent purification steps.

Establishment of PP T Cell Clones. C3H/HeJ mice were given SRBC by gastric intubation for 2 consecutive d, as previously described (13). Mice were killed 1 wk later, their PP aseptically removed, and single-cell suspensions prepared after treatment of PP with Dispase enzyme, as previously described (9, 11). Dissociated PP cells were washed extensively (four to five times) in incomplete RPMI 1640 containing 10% FCS. After the final wash, cells were treated with anti-mouse Ig and rabbit complement (C), as previously described (13, 21). This procedure was repeated two additional times, and the resulting T cells were further purified by separation on a Ficoll-Hypaque gradient. Cell purity was established using fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-Thy-1.2 (Becton, Dickinson & Co., Sunnyvale, CA) and tetramethyl rhodamine isothiocyanate goat anti-mouse Ig ($\kappa + \lambda$). The resulting cell populations consisted of 93–95% Thy-1.2$^+$ and <1% Ig$^+$ cells.

Purified PP T cells were resuspended (1×10^6 cells/ml) in incomplete RPMI 1640 medium containing 10% FCS and cultured in 16-mm multiwell culture plates (Linbro Chemical Co., Hamden, CT) in the presence of IL-2 (2–3 U/ml), SRBC (1×10^6), and feeder cells (see below). Cultures were incubated at 37°C in a humidified chamber containing an atmosphere of 7% O_2, 10% CO_2, and 83% N_2, and supplemented with IL-2 every 3rd d and with fresh feeder cells each week. Wells exhibiting clone growth were expanded into several wells of macroculture plates (usually 2–3 wk after initial culture). Cultures exhibiting good growth (usually within 7–10 d) were subcloned by limiting dilution into 96-well microculture plates, as previously described (19). Subclones were incubated with IL-2 and feeder cells but without antigen. Clones were expanded in macroculture plates and supplemented with IL-2 and feeder cells as described above.

Feeder Cells. Spleens from normal C3H/HeJ mice were dissociated into single cells, washed with incomplete RPMI 1640 medium, and treated with monoclonal anti-mouse Thy-1.2 antibody (a kind gift of Dr. G. J. Hämmerling) and rabbit C. Cells were then washed by centrifugation and the pelleted cells irradiated (1,500 rad). This treatment completely abolished lymphocyte responses to either T or B cell mitogens. To insure that complete inactivation of lymphoid cells in feeder cultures was obtained, pelleted cells were irradiated with 3,000 rad, and this treatment has been used in most of the studies reported here. Cells were washed and resuspended (1×10^6 cell/ml) in incomplete RPMI 1640 medium supplemented with 10% FCS and added (0.1 ml) to macroculture wells. For cloning in microculture plates, a final concentration of 10^4 feeder cells was added to each well.

In Vitro Immune Responses. Single-cell suspensions from either spleen or PP of C3H/HeN mice were treated twice with anti-Thy-1.2 and C and used as B cell cultures. In some experiments, B cell cultures from BALB/c and C57BL/10Sn mice or spleen cell cultures from C3H/HeN and BALB/c nude mice were used. Cells were washed and resuspended in minimal essential medium (MEM; Gibco Laboratories, Grant Island Biological Co.) supplemented with L-glutamine, gentamycin, sodium bicarbonate, sodium pyruvate, nonessential amino acids, and 10% FCS (complete MEM) (13), added to macroculture wells (2.5×10^6 cells/0.25 ml) containing antigen and cloned T cells, and incubated at 37°C, as described above.

PFC Assay. After 5 d of incubation, nonadherent cells were removed from culture wells, washed in Hanks' balanced salt solution (HBSS), and resuspended in HBSS to the appropriate dilutions for bioassay. Cultures were assessed for both direct (IgM) and indirect (IgG_1, IgG_2, and IgA) anti-erythrocyte plaque-forming cell (PFC) responses using the slide modification method described previously (11, 13, 21).

Characteristics of PP T_h A Clones. PP T cell clones that support IgA responses (PP T_h A) were incubated with FITC-labeled anti-Thy-1.2 or with either monoclonal anti-Lyt-1 or anti-Lyt-2 (anti-framework) antibodies followed by TRITC-labeled anti-rat IgG_2 (11). In other experiments, T_h A clones were incubated with either TRITC-goat anti-Ig or FITC-labeled monoclonal IgG_{2_b} anti-I-A^k. Stained cells were enumerated with an immunofluorescence microscope (Orthoplan, Leitz, Wetzlar, West Germany). Depending on the cell number, between 10 to 15 fields were counted (at least 1,200 cells).

For assessment of surface Fc receptors on cloned cells, a direct immunocytoadherence assay with trinitrophenyl (TNP)-SRBC was used (22). Purified IgA anti-dinitrophenyl(DNP) was obtained from MOPC 315 ascites fluid after elution from a TNP-KLH immunoadsorbent column. Monoclonal IgM and IgG_{2_a} anti-DNP were also purified from this column. The purified antibody preparations were titrated by hemagglutination using lightly conjugated TNP-SRBC (13) and used at subhemagglutinating levels in the rosette assay. For rosetting, aliquots of either IgM, IgG_{2a}, or IgA-conjugated TNP-SRBC were mixed with cloned PP T_h A cells, and immunocytoadherence was scored (23).

Statitics. Values for the PFC assay are expressed as the mean PFC response per culture ± SEM. The significance of difference between means was determined by the Student's *t* test.

Results

Assessment of PP Clones for T Helper Activity. PP T cell cultures usually required 2 wk to show significant cell division and growth. Generally, at 2–3 wk after culture initiation, >80% of wells exhibited cell proliferation and visible growth and required subculture. Individual clones were then established by limiting dilution, as described by Watson (19). A cloning efficiency of ~45–55% was seen. We established clones from murine PP derived from animals orally primed with either SRBC, horse erythrocytes (HRBC), or keyhole limpet hemocyanin (KLH). For convenience, only our results with SRBC-specific clones are presented here.

A significant number of clones from murine PP exhibit T_h cell activity (63 of 212 clones tested), and data is presented in Table I for 21 clones exhibiting T_h cell activity for IgA responses (21/63, or 33% of those tested). Generally, clones could be divided into two broad groups, i.e., those supporting IgM and IgA anti-SRBC PFC responses and those that supported low IgM, IgG_1, IgG_2, and high IgA anti-SRBC PFC responses (Table I). The clones could also be further subdivided into those supporting either high (>1,000 IgA PFC/culture) or moderate IgA PFC responses. The distinction in T_h cell activity for IgM and IgA, on the one hand, and for IgM, IgG_1, IgG_2 and IgA, on the other hand, was not due to mixed clones because recloning of T cells in each of these categories yielded progeny with exactly the same properties (data not shown).

Our next experiment was directed to assess T_h cell activity of individual clones for IgA responses under even more stringent conditions, i.e., spleen cell cultures from animals previously primed with SRBC (Table II). Addition of purified splenic T cells from mice systemically primed with antigen to B cell cultures immunized with SRBC gave IgM and largely IgG_1 and IgG_2 responses, clearly indicating the presence of memory B cells for IgG isotype responses (Table II). When cloned T cells from PP were added to these B cell cultures, a similar pattern was seen to that observed for T_h cells in normal B cell cultures. Generally, clones that supported IgM and IgA responses in normal cultures gave comparable responses in B cell cultures from primed animals. Cloned T cells that supported all three isotype responses, but largely IgA responses, also exhibited this capacity in primed B cell cultures. The presence of memory B cells for IgG responses did not favor these isotype responses when cloned PP T_h cells were added. Thus, two broad categories of T_h cells for IgA responses can be distinguished; those that support IgM and IgA and those that support low IgM, IgG_1, and IgG_2 and high IgA anti-SRBC PFC responses. Both categories are therefore considered to be T_h cells for IgA isotype responses (designated PP T_h A clones).

Six PP T_h A clones (1, 5, 7, 9, 11, and 14) were selected for further analysis because each of these clones promoted good IgA anti-SRBC PFC responses. Our past work (11) indicated that, although PP lymphoreticular cells from normal mice support in vitro immune responses to SRBC, IgA responses were seen only in PP cultures from

TABLE I

*Peyer's Patch T Cell Clones Support In Vitro IgA Responses in Normal Splenic B Cell Cultures**

PP T_h A cells added to culture (clone number)	Anti-SRBC PFC/culture‡			
	IgM	IgG_1	IgG_2	IgA
None	3 ± 3	0	0	0
IgM and IgA only				
1	755 ± 40	0	0	2,263 ± 102
5	910 ± 33	0	0	1,911 ± 61
16	1,325 ± 95	0	0	1,450 ± 60
18	1,330 ± 61	0	0	1,495 ± 113
20	1,245 ± 115	0	0	1,885 ± 36
4	697 ± 61	0	0	645 ± 35
8	421 ± 17	0	0	747 ± 19
12	422 ± 17	0	0	347 ± 29
15	605 ± 14	0	0	466 ± 18
IgM, IgG, and IgA				
7	241 ± 18	209 ± 11	164 ± 17	1876 ± 71
9	412 ± 47	705 ± 31	374 ± 28	1893 ± 117
10	187 ± 9	309 ± 17	168 ± 7	1210 ± 78
11	577 ± 42	48 ± 14	0	1755 ± 70
14	635 ± 45	130 ± 8	0	2950 ± 152
17	625 ± 48	25 ± 2	32 ± 8	1030 ± 52
19	345 ± 25	570 ± 19	325 ± 17	1870 ± 105
2	471 ± 61	169 ± 13	102 ± 9	819 ± 43
3	311 ± 41	229 ± 14	106 ± 17	505 ± 19
6	277 ± 19	270 ± 18	162 ± 21	855 ± 41
13	315 ± 22	285 ± 71	202 ± 9	662 ± 25
21	575 ± 5	430 ± 12	129 ± 6	775 ± 16

* Spleen cells were treated with anti-Thy-1.2 and rabbit C and cultured (2.5 × 10^6 cells/well) with SRBC (2–3 × 10^6) and T cells (5 × 10^4 cells/well). Direct and indirect anti-SRBC PFC responses were assessed on day 5 of culture.

‡ Values are the mean anti-SRBC PFC/culture from triplicate cultures per experiment and three separate experiments. Responses of control cultures were: spleen cells + SRBC; IgM, 1682 ± 71; IgG_1, 171 ± 16; IgG_2, 108 ± 21; and IgA, 0. Spleen cell or splenic B cell cultures alone gave no direct or indirect PFC responses.

mice orally primed, suggesting that antigen-primed cells were required for IgA responses. When PP T_h A cells were added to normal PP B cell cultures (Table III) and immunized with SRBC, significant IgA responses were noted. This clearly suggests that T_h cells for IgA responses are of central importance for expression of this isotype, and primed PP B cells are not a significant prerequisite.

Functional Characteristics of Cloned PP T_h A Cells. At the present, cloned T cells from murine PP have been maintained in continuous culture for >30 wk. Clones that exhibit T_h activity have maintained their ability to support IgA responses (Table IV). It is interesting that lines that gave high (1, 3, 14) or moderate (7, 9, 11) IgA responses maintain this property over relatively long periods of time in culture.

Cloned T_h A cells are TCGF dependent and antigen independent (after an initial 2-wk period with antigen) and exhibit good growth in micro- or macroculture wells (Fig. 1). Interestingly, T_h A cell clones for only IgM and IgA isotope responses (T_h A

TABLE II

*Peyer's Patch T Cell Clones Support In Vitro IgA Responses in Primed Splenic B Cell Cultures**

PP T$_h$ A cells added to culture (clone number)	Anti-SRBC PFC/culture‡			
	IgM	IgG$_1$	IgG$_2$	IgA
Control§ (primed T cells)	988 ± 72	3,141 ± 88	2,742 ± 76	79 ± 11
None	15 ± 2	0	0	0
IgM and IgA only				
1	1,852 ± 157	0	0	3,262 ± 97
5	1,762 ± 122	23 ± 11	0	2,272 ± 57
16	820 ± 16	0	0	1,898 ± 52
18	861 ± 15	0	0	1,091 ± 25
20	1,245 ± 115	0	0	1,260 ± 11
4	560 ± 80	0	0	990 ± 17
8	585 ± 25	0	0	510 ± 8
12	620 ± 60	0	0	467 ± 10
15	633 ± 8	0	0	784 ± 33
IgM, IgG and IgA				
7	220 ± 19	160 ± 11	109 ± 13	1,380 ± 12
9	270 ± 11	960 ± 51	872 ± 109	2,121 ± 45
10	337 ± 48	142 ± 72	169 ± 13	589 ± 50
11	511 ± 13	527 ± 41	420 ± 50	1,840 ± 171
14	850 ± 35	65 ± 5	42 ± 11	2,619 ± 21
17	294 ± 11	190 ± 10	116 ± 17	1,179 ± 12
19	1,230 ± 21	210 ± 14	107 ± 11	861 ± 53
2	570 ± 60	240 ± 11	108 ± 7	1,102 ± 37
3	517 ± 52	330 ± 12	171 ± 18	1,252 ± 80
6	380 ± 10	130 ± 7	17 ± 9	970 ± 11
13	407 ± 37	148 ± 14	168 ± 22	310 ± 70
21	540 ± 41	150 ± 11	128 ± 28	249 ± 10

* Spleen cells from mice carrier primed intravenously with SRBC (0.1 ml, 1%) were treated with anti-Thy-1.2 and rabbit C and cultured (2.5 × 10^6 cells/well) with SRBC (2–3 × 10^6) and T cells (5 × 10^4 cells/well). Direct and indirect anti-SRBC PFC responses were assessed on day 5 of culture.

‡ Values are the mean anti-SRBC PFC per culture from triplicate cultures per experiment and three separate experiments. Responses of control cultures were primed spleen cells + SRBC; IgM, 1,089 ± 21; IgG$_1$, 1,247 ± 62; IgG$_2$, 889 ± 76; and IgA, 108 ± 19. Primed spleen cells or splenic B cell cultures alone (without antigen) gave no direct or indirect PFC responses.

§ Purified splenic T cells from mice carrier primed with SRBC.

1 and 5) exhibited higher growth rates than clones supporting low IgM and IgG and high IgA responses (T$_h$ A 7 and 9) (Fig. 1). This has been a consistent finding with all 21 clones tested thus far (data not shown). Addition of excess TCGF or more purified IL-2 to T$_h$ A clones supporting all three isotype responses did not significantly increase their rate of division (approximately one division every 36 h).

All PP T$_h$ A clones tested are antigen specific and support in vitro IgA responses only in the presence of the homologous antigen, SRBC (Fig. 2). Cloned T$_h$ A cells do not support in vitro immune responses of B cell cultures immunized with HRBC or chicken erythrocytes (Fig. 2).

Previous studies with cloned T$_h$ cells suggested a stringent requirement for H-2 compatibility (19) for effective T cell help. Our experiments also clearly indicate that

TABLE III

*Peyer's Patch T Cell Clones Support In Vitro IgA Responses in Normal PP B Cell Cultures**

PP T_h A (clone number)	IgA anti-SRBC PFC/culture
1	5,326 ± 186‡
5	4,742 ± 108
7	2,849 ± 64
9	3,148 ± 103
11	2,929 ± 66
14	5,542 ± 47
None	0

* Enzyme-dissociated PP cells were treated with anti-Thy-1.2 and C and cultured (2.5×10^6 cells/well) with SRBC ($2\text{-}3 \times 10^6$) and T_h A cells (5×10^4 cells/well). IgA anti-SRBC PFC were assessed 5 d later.

‡ Values are the mean IgA anti-SRBC PFC response per culture ± SEM from triplicate cultures and two separate experiments.

TABLE IV

*Stability of Peyer's Patch T_h A Clones for Support of IgA Responses**

PP T_h A cells added to B cell cultures (clone number)	IgA anti-SRBC PFC responses/culture (age of clone in culture in weeks)‡				
	3	5	10	14	24
1	2,462 ± 108	2,610 ± 28	3,262 ± 97	2,819 ± 51	3,172 ± 84
5	2,347 ± 18	2,272 ± 57	1,765 ± 19	2,378 ± 71	2,016 ± 32
7	1,182 ± 18	1,380 ± 12	1,032 ± 22	1,424 ± 43	1,721 ± 71
9	1,210 ± 17	1,100 ± 45	1,431 ± 11	1,285 ± 24	1,471 ± 62
11	779 ± 42	840 ± 17	1,043 ± 26	791 ± 21	947 ± 33
14	3,095 ± 82	2,721 ± 92	3,171 ± 103	2,950 ± 152	3,475 ± 80

* PP T_h A cells were harvested after varying numbers of weeks in culture, added (5×10^4 cells/well) to primed splenic B cells (2.5×10^6 cells/well), and incubated with SRBC ($2\text{-}3 \times 10^6$). IgA anti-SRBC PFC responses were assessed on day 5 of culture.

‡ Values are the mean anti-SRBC PFC per culture from triplicate cultures per experiment.

H-2 specificity is required for T_h cell promotion of IgA isotype responses (Table V). T_h A clones supported IgA responses in C3H/HeN nude splenic or B cell cultures but not in cell cultures derived from H-2-incompatible mice (Table V). Thus, complete H-2 compatibility is required for efficient cell interactions involved in the IgA response. Failure of T_h A cell clones to support immune responses in H-2^b or H-2^d B cell cultures was not because of the absence of histocompatible antigen-presenting accessory cells, because co-culture of T_h A cells with H-2^k-irradiated feeder cells and H-2^b and H-2^d B cell cultures also yielded poor IgA responses (data not shown).

Surface Phenotype of Cloned PP T_h A Cells. All PP T_h A cell clones tested thus far are Thy-1.2^+ and Lyt-2^- (Table VI). In addition, T_h A cells are Lyt-1^+; however, a different pattern of surface staining is seen between cloned T cells and normal splenic or PP T cells examined for surface Lyt-1 antigen. Cloned T_h A cells exhibited a complete halo of dull fluorescence, whereas normal splenic or PP Lyt-1^+ T cells exhibit strong, patchy surface immunofluorescence. None of the clones examined thus far exhibit surface Ig or I-A (Table VI).

Previous studies have shown the existence of both murine (23) and human (24) T

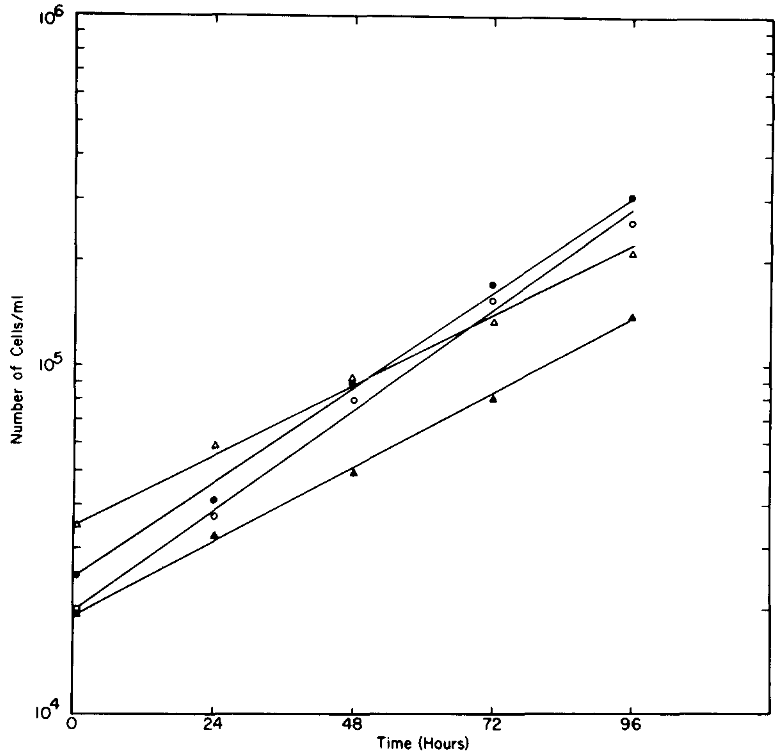

FIG. 1. Growth characteristics of cloned PP T_h A cells. Cells were grown in microculture wells in complete media containing TCGF, harvested at daily intervals, and viable cells (>95%) enumerated. Individual clones assessed were T_h A 1 (○), 5 (●), 7 (△), and 9 (▲).

cell subpopulations that form rosettes with IgA-coated erythrocytes, clearly suggesting the presence of T cell subpopulations with Fc receptors for IgA. We tested this property with cloned T_h A cells (Table VII). No IgM or IgG_{2a} rosetting occurred with PP T_h A cell clones; however, the majority of T_h A cells formed rosettes with IgA-coated erythrocytes. These results clearly suggest that PP T_h A cells bear surface Fc receptors for IgA. In other studies using FITC-labeled MOPC 315 IgA, >99% of T_h A clones were positive by immunofluorescence or by fluorescence-activated cell sorter (FACS) analysis.

Discussion

Clones of T cells have been derived from murine PP, which support the IgA immune response (PP T_hA). All of these cloned cells are Thy-1.2^+, Lyt-1^+, Lyt-2^-, and possess Fc receptors for IgA (FcR_α), are antigen specific for T_h A activity, and require full H-2 compatibility. Two broad groups of T_h A cell clones have been derived. One group supports some IgM and largely IgA responses in murine B cell cultures, and a second group promotes small but distinct IgM, IgG_1, and IgG_2 and large IgA responses. Thus, we obtained compelling evidence for T cells that predominantly help the IgA response.

In recent years, several laboratories have presented evidence that IgA responses are thymic dependent and are regulated by unique populations of T lymphocytes in

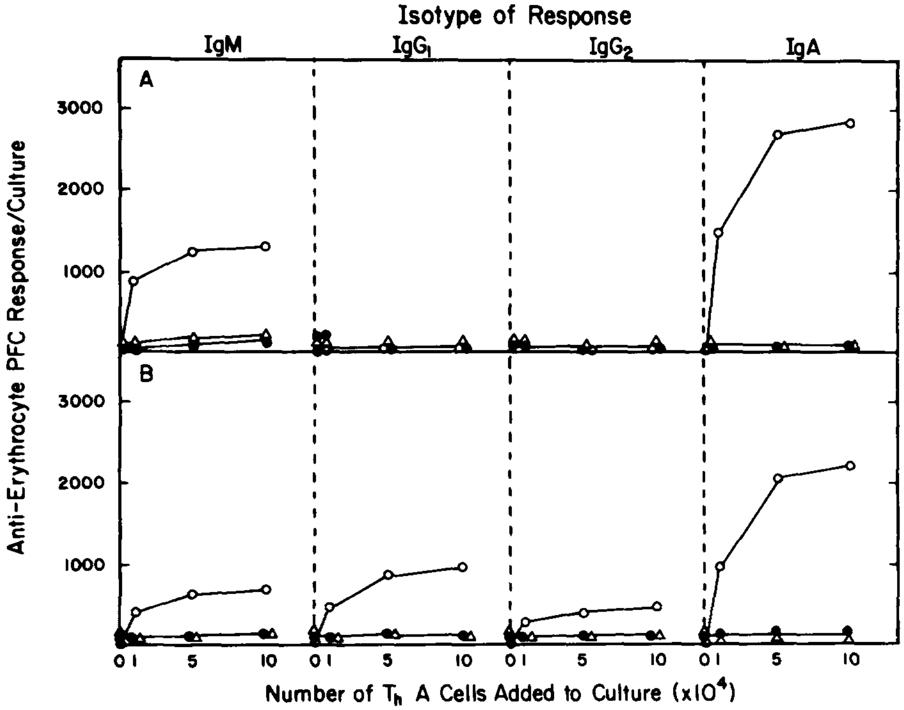

FIG. 2. Helper activity of T_h A clones for IgM, IgG$_1$, IgG$_2$, and IgA isotype responses. Graded doses of either T_h A 1 (A) or T_h A 9 (B) were added to C3H/HeN splenic B cell cultures immunized with either SRBC (○), HRBC (●), or CRBC (△). The isotype (IgM, IgG$_1$, IgG$_2$, or IgA) of anti-erthrocyte PFC response was determined on day 5 of culture.

TABLE V

H-2 Specificity Is Required for T Cell Help in the IgA Response

Source of spleen cells*	IgA anti-SRBC PFC/culture (PP T_h A clone number)‡	
	1	9
nu/nu		
BALB/c	232 ± 17	187 ± 21
C3H/HeN	2,487 ± 93	2,292 ± 104
B cell culture		
BALB/c	137 ± 7	155 ± 18
C57BL/10Sn	102 ± 8	121 ± 17
C3H/HeN	2,662 ± 48	2,171 ± 88

* Spleen cells (nude) (2.5×10^6 cells/well) or purified splenic B cells (treated with anti-Thy-1.2 and C; 2.5×10^6 cells/well) were cultured with SRBC (2–3×10^6) and T_h A cloned cells (5×10^4 cells/well). Cultures were bioassayed on day 5 for IgA anti-SRBC responses.

‡ Values are the mean IgA anti-SRBC PFC response per culture ± SEM from triplicate cultures and four separate experiments. Responses of control cultures (no T cells added) were nude (BALB/c, 4; C3H/HeN, 8) or B cell cultures (BALB/c, 7; C57BL/10Sn, 6; C3H/HeN, 10).

TABLE VI

Characterization of PP T_h *A Clones for Cell Surface Antigens**

PP T_h A clone number	Percentage of cells bearing antigen‡				
	Thy-1.2	Lyt-1	Lyt-2	I-A^k	Ig(K + λ)
1	>99	99.5	0	0	0
5	>99	97.0	0	0	0
7	>99	97.0	0	0	0
9	>99	98.0	0	0	0
11	>99	93.5	0	0	0
14	>99	98.0	0	0	0

* Triplicate slides were prepared from each PP T_h A cell clone, and the number of positive cells was enumerated in 10–15 fields. At least 1,200 cells were scored for each slide per experiment.

‡ Values are the mean percentage of three separate experiments.

TABLE VII

Isotype of Fc Receptors Present on PP T_h *A Cells**

PP T_h A clone number	Percentage of rosette formation‡		
	IgM	IgG2a	IgA
1	<1	0	88.0
5	<1	0	90.0
7	0	0	91.5
9	0	0	96.4
11	<1	0	96.5
14	0	0	89.0

* Monoclonal IgM (κ), IgG_{2_a} (κ), or IgA (λ) anti-DNP antibodies were reacted with TNP-SRBC followed by incubation with PP T_h A cells. The number of rosettes was enumerated in 10–15 fields. At least 1,500 lymphoid cells were scored for each slide per experiment.

‡ Values are the mean percentage of two separate experiments.

lymphoid tissue, e.g., murine PP, where induction of IgA responses principally occur. Strober and associates (23, 24) first reported that a subset of T cells from either mouse spleen (23) or human peripheral blood (24) bear Fc receptors for mouse IgA. In further studies, Gebel et al. (22) and Lynch and Hoover (25) found a high percentage of peripheral blood lymphocytes bearing myeloma IgA in mice carrying an IgA plasmacytoma cell line, clearly suggesting that secreted myeloma IgA induced Fc_α receptors on lymphoid cells. This has been corroborated by recent in vitro studies (26) that showed that polymeric IgA induced FcR_α expression on both T and B lymphocytes. Although FcR_α T cells purified by rosetting with IgA-coated erythrocytes generally do not selectively support IgA responses in vitro, recent evidence has been presented that separation of FcR_α cells by FACS from human peripheral blood yields a T cell subpopulation that preferentially supports IgA (and some IgM and IgG) synthesis in B cell cultures stimulated with pokeweed mitogen (27). The present experiments verify the conclusions of this former study that T_h cells for IgA responses bear FcR_α.

The present study provides compelling evidence that a unique population of T_h cells are involved in the IgA response. Cloned T_h cells from murine PP have been propagated in continuous culture without affecting their ability to promote IgA responses. In fact, cloned T cells maintained an ability to either support IgM and largely IgA responses or to support IgM, IgG_1, IgG_2, and largely IgA responses. Thus far, these two groups of T_h A clones can only be distinguished by growth characteristics in culture or their function in isotype response. T_h A cells for IgM and high IgA responses divide once every 24–26 h (Fig. 1), whereas T_h A cells that support low IgG responses exhibit doubling times of ~36 h. Neither group bears significant I-A or Ig and show identical surface staining for Thy-1.2 and Lyt-1 antigens. The diffuse staining observed for Lyt-1 is different from that observed on normal PP or splenic T cells (bright patchy staining of ~60% of all T cells) and may be a characteristic of long-term cultures of T_h cells. The significance of T cell help for small IgG subclass responses is not known; however, this appears to be a minor characteristic of this cell type because T_h A clones in this group did not support markedly higher IgG_1 or IgG_2 isotype responses in primed B cell cultures when compared with in vivo induced splenic T_h cells (Table II). An equally plausible explanation would be that the T_h A cells promote switching from IgG to IgA responses. Nevertheless, this minor help for IgG subclass responses was not due to contaminating T_h cells for IgG responses in the clone because each clone was derived from a single cell by limiting dilution. Furthermore, recloning of T_h A cells resulted in subclones that exhibited identical properties of the parent clone, e.g., T_h A clones for IgM and high IgA isotype responses, after recloning, supported only these same isotypes, whereas T_h A clones supporting low IgG_1 and IgG_2 responses, when subcloned, exhibited the same characteristic. Thus, the first group may facilitate an IgM → IgA switch, whereas the second group may promote IgM → IgG → IgA responses. If this is true, the PP T_h A cells that support IgG_1 and IgG_2 should also support IgG_3, whereas PP T_h A clones for IgM and IgA only should not. We are currently testing this hypothesis, and the results will be reported in a separate communication.

Recent studies (28, 29) have shown that antigen-activated T_h cells can also expand B cell populations in a polyclonal fashion. Although we have not directly studied this in mitogen-driven B cell cultures, we have consistently observed that all 21 T_h A clones extensively studied to date are quite antigen specific. These T_h A clones will not support in vitro immune responses to either HRBC or chicken erythrocyte, but promote IgA responses in B cell cultures immunized with SRBC. When T_h A cells were incubated with B cell cultures without antigen no significant mitogenic, polyclonal or immune responses were seen (data not shown).

Our past studies (11, 13) and those of others (12) have shown that significant T_h cell activity can be induced in murine PP by oral administration of TD antigen. The present study clearly indicates that these T_h cells can be clonally expanded in vitro and maintained in culture for extended periods without loss of helper activity. Our studies would also indicate that a high frequency of T_h cells induced in murine PP, preferentially, but not exclusively, supports the IgA response. Nevertheless, it is possible that T_h cells for other isotype responses also arise in PP, and these cells may not be amenable to clonal expansion in vitro. Alternatively, these putative T_h cells may emigrate rapidly out of GALT to the periphery.

The finding that T_h A clones promote elevated IgA PFC responses in PP B cell cultures (Table III) further corroborates the importance of GALT as inductive sites for the IgA response. At present, we do not understand why full collaboration for IgA responses fails to occur in the PP itself but is instead manifested after sensitized cells have migrated to distant mucosal sites. One distinct possibility would be the simultaneous induction of T suppressor (T_s) cells in GALT that would act either on T_h A cells or IgA precursor B cells to prevent local immune responses. In this regard, it is well known that T_s cells can specifically inhibit IgG responses (30). In some instances, T_s cells may negatively regulate expression of IgG subclasses (31). Some evidence for IgA isotype-specific T_s cells has been derived from studies with IgA deficient subjects (6). We are currently exploring this possibility by isolation and growth of T_s cells from murine PP, and cloned T_s cells will be tested for isotype-specific suppression, either at the level of T_h A cells or directly on precursor IgA B cells.

Results of the present study have a direct bearing on studies of others that have suggested that T_h cells require collaboration among T cell subsets for induction of B cell responses. Tada and co-workers (32) and Marrack and Kappler (33, 34) have provided evidence for the occurrence of two distinct subsets of T_h cells. These cells have distinct surface recognition receptors and provide two separate signals for induction of B cell responses. In more recent studies (35), the latter group has shown that two distinct factors, one of which is IL-2 (TCGF) and the other presumed IL-3, are both required by B cells for specific responses to TD antigens. This model is not precluded by our results for IgA responses because T_h A clones are both dependent upon TCGF for growth and perhaps produce IL-2 in culture. Furthermore, the T_h A clone produces a second factor for IgA B cell responses. In studies to be published, we found that culture supernatants from T_h A clones grown with TCGF and SRBC produce soluble factor(s) that support IgA responses when added to B cell cultures immunized with antigen. Purified IL-2 added to B cell cultures supports only IgM PFC responses (manuscript in preparation). Further studies will be required to determine whether IL-3 and IgA helper factor are distinct entities and whether IgA factor alone is sufficient for directing B cells to IgA synthesis. Studies along these lines are currently in progress.

It is now established that T_h cells promote B cell differentiation to antibody synthesis through recognition of both antigen (antigen-specific) and cell surface major histocompatibility complex (MHC) products on T_h cells and syngeneic B cells (36). Elegant recent studies of Pierce and her collaborators (37–39) have further shown that B cells from immune or nonimmune mice differ in MHC requirements in T cell collaboration for individual isotype expression. Secondary (immune) B cells require completely syngeneic MHC recognition by T cells, whereas primary B cells are less stringent for production of IgM antibody (39). The acquisition of the secondary B cell MHC collaborative phenotype is dependent upon T cells. Thus, although antigen in the absence of T cells induces B cell precursors for IgG_1 isotype responses, these B cells do not express proper MHC for IgG_1 responses. On the other hand, induction in the presence of T cells results in full MHC expression for IgG_1 synthesis (39). Our results with T_h A clones fully support previous evidence for full MHC compatibility for secondary responses. However, we observe IgA isotype-specific responses in nonprimed B cells. We cannot, of course, preclude that prior exposure of lymphoid cells to environmentally related antigens has occurred in normal mice; future experiments will use B cell cultures derived from germ-free C3H/HeN mice to which T_h A cells have been added to fully address this point.

There are striking similarities between T cell regulation of IgA responses and studies of others (40) that have shown T cell-directed IgE isotype specific responses. Recent extensive studies of Ishizaka and co-workers (41–45) have shown that administration of either complete Freund's adjuvant (CFA) or *Bordetella pertussis* vaccine (BP) to rats induces T cell subpopulations that release IgE-binding factors. T cells from BP-treated rats release an IgE-binding factor that enhances the IgE response (44), whereas T cell factors from CFA-treated rats bind IgE and suppress the response (45). Potentiation of IgE responses also requires adherent cells (MØ) that produce an interferon-like inducer (45). The MØ inducer results in production by T cells of incomplete IgE-binding factor. A subset of T cells from BP-treated rats produces soluble factors that enhance glycosylation of IgE-binding factor, and the latter factor then becomes an active potentiator for IgE responses (44, 45). On the other hand, T_s cells from CFA-treated rats produce inhibitors of glycosylation, and the subsequent nonglycosylated IgE-binding factor suppresses IgE responses (45). From our studies, it is clear that T_h A cells exhibit Fc_α receptors; however, we still do not know whether IgA factor(s) produced by these cells bind IgA. Experiments along these lines are currently underway.

Summary

We successfully cloned antigen-specific T cells from murine gut-associated lymphoreticular tissue, i.e., Peyer's patches, which are dependent upon T cell growth factor and independent of antigen for continuous growth. These clones exhibit helper activity for IgA responses to sheep erythrocytes (SRBC) and have been designated T helper (T_h) A. Two broad categories of T_h A clones have been maintained in continuous culture. The first group supports IgM and largely IgA anti-SRBC plaque-forming cell (PFC) responses in both normal and SRBC-primed splenic B cell cultures, whereas the second group supports low IgM, IgG_1, and IgG_2 and high IgA PFC responses. Subclones derived from single cells maintain the parent helper properties when propogated in culture for long periods (>7 mo). Cloned T_h A cells are antigen specific and do not support polyclonal or immune responses to other thymus dependent antigens in normal B cell cultures. T_h A cells require full histocompatibility for helper functions because addition of cloned T_h A cells to B cell cultures from other H-2 types does not result in IgA responses.

Cloned T_h A cells are Thy-1.2^+ and Lyt-1^+ and Lyt-2^-, Ig^-, and I-A^-. T_h A cells bear Fc receptors for IgA and do not possess receptors for IgM or IgG isotypes. Thus, T cells that primarily promote IgA isotype responses have been isolated in high frequency from murine PP, an anatomical site of major importance for induction and regulation of the IgA response.

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