ANTIGEN-SPECIFIC SUPPRESSION OF HUMAN ANTIBODY RESPONSES BY ALLOGENEIC T CELLS

I. Frequency and Antigen Specificity of Allogeneic Suppressor T Cells and their Role in Major Histocompatibility Complex-controlled Genetic Restriction

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Suppressor T (Ts)[1] cells are known to be involved in the regulation of most immune responses, including antibody production (1, 2), delayed-type hypersensitivity (3), cytotoxicity (4), and responses to mitogens (5). In addition, Ts activated by antigens coded for by the major histocompatibility complex (MHC) (allogeneic Ts) have been found to be important in the regulation of various allogeneic responses. Thus, allogeneic Ts are known to inhibit mixed lymphocyte responses (6) and, in vivo, they are probably responsible for the loss of immune competence in animals undergoing graft-vs.-host (GvH) reactions (7, 8). Similarly, allogeneic Ts activated in pregnancy inhibit the generation of cytotoxic T cells (CTL) to paternal antigens and may be important for maternal tolerance of the fetus (9, 10).

One of the more successful means for studying allogeneic suppression has been the potent inhibition of in vitro antibody responses by histoincompatible T cells (11, 12). With this model, allogeneic Ts in both mouse and man have been shown to be radiosensitive T cells belonging to the suppressor/cytotoxic subset with the surface phenotype Ly-1$^+$2$^+$ and Leu-2a$^+$, respectively (11, 13–16). They are, therefore, distinct from T cells mediating positive allogeneic effects that are radioresistant and Ly-1$^+$2$^-$ (17). Gene-mapping experiments have shown that allogeneic Ts are activated by differences at the MHC (18, 19).[2] Potent suppression can be elicited by differences at a single class I locus (11, 18, 19), whereas class II antigens have a smaller effect, or no effect at all (11). Moreover, combined

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[1] *Abbreviations used in this paper:* AET, S-2-aminoethylisothiouronium bromide hydrobromide; CTL, cytotoxic T lymphocytes; DTH, delayed-type hypersensitivity; EIA, enzyme immunoassay; E$^+$, mononuclear cells forming rosettes with AET-treated sheep red cells; E$^-$, non-rosette-forming cells; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; GvH, graft-vs.-host; MHC, major histocompatibility complex; MIg, mouse immunoglobulin; PBM, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PWM, pokeweed mitogen; SN, supernatant; Th, T helper cells; Ts, T suppressor cells.

[2] Callard, R. E., S. L. Tiernan, and C. M. Winger. Antigen-specific suppression of human antibody responses by allogeneic T cells. II. MHC and cell-cell interaction requirements. Manuscript in preparation.

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class I and II differences do not result in enhanced suppression (11), indicating that the response does not require Ts inducer cells, which are normally class I restricted, and that suppression is not mediated by alloactivated CTL.

Despite the considerable amount of work already done on allogeneic Ts, the mechanism by which they suppress in vitro antibody production is unknown. In theory, suppression could occur for rather trivial reasons such as consumption of essential growth factors by alloactivated T cells (20). On the other hand, it is possible that normal, antigen-specific Ts are activated by exposure to alloantigens. In the present study, allogeneic T cells were shown to suppress in vitro antibody responses by human blood lymphocytes in an antigen-specific manner, thereby supporting the notion that allogeneic suppression is mediated by antigen-specific, or possibly idiotype-specific, Ts. In addition, the depletion of Leu-2a$^+$ cells abrogated suppression and permitted T cell collaboration with histoincompatible B cells in T cell-dependent antibody responses.

Materials and Methods

Cell Preparation. Peripheral blood mononuclear cells (PBM) were obtained by centrifugation of heparinized venous blood over Ficoll-Hypaque ($\rho = 1.077$ kg/l^{-1}). The interface (mononuclear) cells were collected and washed three times in RPMI 1640 medium containing 10 mM Hepes and 5% fetal calf serum (FCS). Sheep erythrocyte rosette-forming cells (E$^+$) were formed with aminoethyl isothiouronium bromide hydrobromide (AET)-treated sheep red blood cells at 4°C (21) and separated from non–rosette-forming cells (E$^-$) by centrifugation over Percoll ($\rho = 1.080$ kg/l) (14). HLA-A, -B, -C, and -DR typing of PBM was carried out by Dr. J. Bodmer, Imperial Cancer Research Fund, United Kingdom and by Dr. H. Bashir, Australian Red Cross Blood Bank, Sydney.

Antibodies. Leu-2a and -3a antibodies were kindly provided by Becton, Dickinson & Co., Mountain View, CA. Goat anti-mouse Ig (anti-MIg) used in panning experiments was purchased from Tago, Inc., Burlingame, CA. Fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse Ig (FITC sheep anti-MIg) was prepared by Dr. Peter Beverley from affinity-purified and human Ig-absorbed sheep anti-MIg.

Fluorescence-activated Cell Sorting and Analyses. Lymphocytes were stained for 30 min on ice with saturating amounts of monoclonal antibody diluted in Hepes-buffered medium containing 5% FCS. After three washes, the bound antibody was detected with FITC sheep anti-MIg. To avoid Fc binding, a small amount of normal sheep serum was included in the washing and incubation medium as described previously (22). Cells were analyzed and sorted on a FACS IV (B-D FACS Systems, Becton, Dickinson & Co., Sunnyvale, CA).

Panning. Mononuclear cell subsets coated with monoclonal antibody were also fractionated by a panning technique (23). Plastic petri dishes (100 × 25 mm, No. 4026; Lab-Tek Div., Miles Laboratories, Inc., Naperville, IL) were coated with goat anti-MIg at 10 mg/ml in phosphate-buffered saline (PBS) for 60 min at 4°C and then washed three times with PBS. PBM were incubated with saturating amounts of monoclonal antibody for 30 min at 4°C, washed, and then incubated for 70 min at 4°C on the anti-MIg–coated petri dishes. Between 20 and 30 × 10^6 cells in 10 ml of Hepes-buffered RPMI 1640 containing 5% FCS were incubated on each dish. Nonadherent cells were then removed by pipette and the dish washed three times with medium. Further medium was then added and the adherent cells removed by vigorous pipetting. Purity of the separated populations was generally between 95 and 98%.

Antibody Formation In Vitro. For specific antibody responses, 2 × 10^6 PBM were cultured in 1 ml of RPMI 1640 containing 25 mM Hepes (Gibco Laboratories, Grand Island, NY) and 10% horse serum (CSL, Victoria, Australia) in 12 × 75-mm capped Falcon tubes (Falcon Labware, Oxnard, CA) as described previously (24). Purified influenza virus strains A/X31 (A/H3N2) and B/HK (B-8/73) (kindly supplied by Dr. J. Skehel and Dr. J. Downie) were added at optimal concentrations determined in preliminary experiments,

usually ~1 μg/ml. Cultures were incubated at 37°C for 6 d in an atmosphere of 5% CO_2 in air. At the completion of the culture period, the cells were washed twice, resuspended in 0.5 ml of Hepes-buffered RPMI 1640 containing 5% FCS, and incubated for a further 24 h at 37°C in air. Culture supernatants (SN) were then collected and stored at −20°C before the assay for antibody to influenza. For limiting dilution assays, antibody-forming cultures were carried out in round-bottomed microtiter trays (25, 26). Control cultures contained 4×10^5 PBM per well. Otherwise, 1.5×10^5 E^- cells per well were recombined with 2.0×10^5 irradiated (1,500 rad) autologous E^+ (helper) cells and various numbers ($0.06–2.0 \times 10^5$) of allogeneic E^+ (suppressor) cells.

Antibody Assay. Specific antibody in culture SN was determined by solid-phase enzyme immunoassay (EIA) as described previously (26).

Analysis of Results. A Poisson distribution analysis was performed on the limiting dilution of Ts cells (27). Results were expressed as a semi-log plot of the percentage of positive cultures (i.e., cultures not suppressed) against the number of added E^+ cells. Regression curves were drawn by least squares fit and r^2 values were calculated. A culture was scored as showing suppression if the SN contained less antibody determined by the EIA than the mean of the replicate control cultures (E^- plus irradiated autologous E^+ [T helper] cells minus the lower 95% confidence limit [$SE \times t^n0.05$]). Control cultures ranged from about 20 to 200 ng/ml depending on the donor. Ts frequencies were calculated as described previously (25, 27).

Results

Suppression of Antibody Production by Allogeneic T Cells. Addition of allogeneic E^+ cells to cultures of PBM stimulated with antigen profoundly suppressed specific antibody production (Table I). The suppressor activity of allogeneic E^+ cells was radiosensitive and was completely ablated with 1,500 rad. Autologous E^+ cells, on the other hand, either had little effect or enhanced antibody production, showing that suppression was not simply due to overcrowding of the cultures.

Allogeneic Ts are Antigen Specific. Suppression of specific antibody responses by allogeneic T cells may be explained in two ways. First, allogeneic interactions could result in the activation of normal, antigen-specific Ts by an inappropriate or nonphysiological pathway similar to that described by Bromberg et al. (28) for activation of delayed-type hypersensitivity (DTH) Ts by allogeneic I-J. Alternatively, suppression may result from nonspecific allogeneic effects such as the consumption of essential growth factors by alloactivated cells. One way of

TABLE I

Allogeneic E^+ Cells Suppress Specific Antibody Production by PBM

Responding cells	E^+ cells added	Antibody response to X31		
		Exp. 1	Exp. 2	Exp. 3
			ng/ml	
PBM (unstimulated)	None	0	5 ± 4	1
PBM	None	26 ± 4	190 ± 40	45 ± 18
PBM	Autologous	31 ± 5	260 ± 73	ND*
PBM	Autologous‡	44 ± 4	419 ± 59	ND
PBM	Allogeneic	0	9 ± 3	4 ± 1
PBM	Allogeneic‡	33 ± 4	384 ± 100	56 ± 14

* Not done.

‡ 1,500 rad irradiation.

distinguishing between these two possibilities is to determine whether or not suppression is antigen specific. This question was approached in three different ways. To begin with, the effect of allogeneic E^+ cells on specific antibody production to X31 and polyclonal Ig synthesis in response to pokeweed mitogen (PWM) was compared (Table II). The response to PWM was measured both as specific antibody to X31 (ng/ml) and as total Ig (μg/ml). In neither case was the response to PWM suppressed by the addition of allogeneic E^+ cells. By contrast, specific antibody produced in response to antigenic (X31) challenge was profoundly suppressed by allogeneic, but not autologous E^+ cells. This result is inconsistent with inhibition by nonspecific allogeneic effects, which would be expected to affect both PWM and antigen responses, and suggests that allogeneic suppression may be due to specific Ts. This conclusion was further supported by a series of limiting dilution experiments in which the frequency of allogeneic Ts was determined by Poisson distribution analyses of the antibody responses obtained by adding a range (0.625×10^4 to 20×10^4) of allogeneic E^+ cells to microcultures of 1.5×10^5 E^- cells plus 2.0×10^5 irradiated autologous E^+ T helper (Th) cells and A/X31 antigen. Control cultures of 1.5×10^{-5} E^- plus 2.0×10^{-5} irradiated autologous E^+ (Th) cells and antigen, but with no added allogeneic E^+ (Ts) cells, were set up to establish the level and range of positive responses. 20 sample wells were assayed at each allogeneic E^+ dilution. A culture was considered to be suppressed if the antibody response was significantly less than the mean of the control response minus the lower 95% confidence level. The result of one such experiment is shown in Fig. 1 as a semi-log plot of the percentage of positive wells (i.e., no suppression) against the number of added allogeneic E^+ cells. A straight line was drawn from a least squares analysis with a correlation coefficient of 0.96. In this example, the frequency of Ts cells was 1.7×10^{-5}. In a series of such experiments, the frequency of allogeneic Ts ranged from 0.8×10^{-5} to 4.5×10^{-5} (Table III).

The frequency of allogeneic Ts determined in these experiments was much

TABLE II

Suppression of Specific, But Not PWM-induced Polyclonal Antibody Responses by Allogeneic E^+ Cells

Stimulus*	Added E^+ cells	Antibody response			
		Exp. 1		Exp. 2	
		Anti-X31‡	Total Ig§	Anti-X31	Total Ig
None	None	1	1	0	1
PWM	None	78 ± 17	22 ± 7	18 ± 5	28 ± 1
PWM	Autologous	64 ± 11	17 ± 2	29 ± 10	34 ± 1
PWM	Allogeneic	56 ± 7	24 ± 4	14 ± 6	27 ± 4
A/X31	Autologous	ND‖	ND	46 ± 10	0¶
A/X31	Allogeneic	ND	ND	4 ± 2	0

* PBM cultures were stimulated wth either PWM or influenza virus (A/X31).
‡ Specific antibody for X31 induced by either PWM or antigen was assayed by EIA and expressed as ng/ml ± 1 SEM.
§ Total Ig was assayed by competition EIA and expressed as μg/ml ± 1 SEM.
‖ Not done.
¶ Not detectable above background.

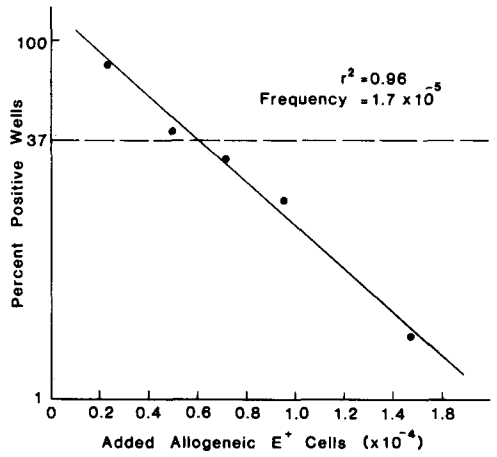

FIGURE 1. Determination of the frequency of allogeneic Ts cells. Various numbers of allogeneic E^+ cells were added to cultures containing 1.5×10^{-5} E^- cells plus 2.0×10^5 irradiated autologous E^+ (Th) cells and stimulated with an optimal dose of X31 influenza virus. A semi-log plot of the percent of responding (nonsuppressed) wells vs. the number of added allogeneic E^+ cells was drawn by least squares fit.

TABLE III

Range of Frequencies of Allogeneic Ts Cells in Antibody Responses to A/X31 Virus

Responder	Allogeneic Ts donor	Frequency of allogeneic Ts ($\times 10^{-5}$)
DW	AE	1.4*
LW	CS	4.5
CS	LW	1.7
RS	DM	1.5
DM	DW	1.2
DM	RS	0.8

* Frequency of allogeneic Ts obtained from semi-log plots of the percent of positive wells vs. the number of added allogeneic E^+ cells drawn by least squares fit (see Fig. 1).

lower than that expected for alloreactive cells, but was within the range expected for antigen-specific cells (29). If suppression by allogeneic T cells is antigen specific, it should be possible to segregate Ts to two non–cross-reacting antigens in limiting dilution cultures. To investigate this, limiting numbers of allogeneic E^+ cells were added to cultures of 1.5×10^{-5} E^- cells plus 2.0×10^5 irradiated autologous E^+ (Th) cells stimulated with either A/X31, B/HK, or both antigens together. At limiting numbers of allogeneic suppressor cells, some wells stimulated with both antigens were suppressed for the production of antibody to only one (Table IV). Moreover, by determining the frequency of allogeneic Ts to both antigens, it was possible to predict the frequency of wells in which antibody production to both antigens should have been suppressed. A χ^2 analysis showed that the observed and expected frequencies of doubly suppressed culture wells were not significantly different (Table V). The complete segregation of suppres-

TABLE IV

Antigen Specificity of Allogeneic Ts Cells Exhibited, at Limiting Dilutions of ALLO E^+, by Suppression of Antibody Responses to One of Two Non–Cross-Reacting Antigens Present in the Same Culture Wells

Allogeneic E^+ dilution	Antibody response to influenza X31/BHK* in culture well number:									
	1	2	3	4	5	6	7	8	9	10
1.5×10^5	14/39‡	16/50	17/9	51/18	27/50	123/199	100/55	56/47	15/46	65/197
0.75×10^5	26/206	120/208	60/41	110/212	115/49	70/64	17/101	70/43	124/54	76/65
0.375×10^5	31/290	24/233	23/73	112/50	120/13	120/157	124/49	51/370	101/350	106/160

* The antibody response to A/X31 and B/HK was calculated as a percentage of the response in control cultures.

‡ Response to X31/BHK. Underlined values indicate wells in which the antibody response to one virus was suppressed while the response to the other virus was not.

TABLE V

Expected and Observed Frequencies of Culture Wells in which the Response to Both A/X31 and B/HK Was Suppressed by Allogeneic E^+ Cells

Exp.	Allogeneic E^+ cells/well ($\times 10^{-5}$)	Percent X31 suppressed	Percent B/HK suppressed	Percent double-suppressed wells	
				Expected	Observed
1	5	90	50	45	40
2	5	60	70	42	40
3	5	50	40	20	20
4	5	50	80	40	50
5	5	80	80	64	60
6	7.5	60	50	30	30
7	5	30	10	3	10
8	5	40	30	12	10
9	5	40	90	36	20
10	5	50	50	25	30

A χ^2 analysis showed that the expected and observed frequencies were not significantly different ($P > 0.5$).

sion to two non–cross-reacting antigens in these experiments shows that allogeneic T cell suppression is antigen specific.

Phenotype of Allogeneic Ts. In earlier work (15), we showed that allogeneic Ts belonged to the Leu-$2a^+$ (suppressor/cytotoxic) subset. These experiments were repeated here using Leu-$2a^+$ cells prepared by panning or FACS sorting. Antibody production in microcultures of 4×10^5 PBM and antigen was profoundly suppressed by the addition of 2×10^5 allogeneic E^+ cells or allogeneic Leu-$2a^+$ ($3a^-$) cells, but not Leu-$2a^-$ ($3a^+$) cells (Table VI). Addition of autologous E^+ cells had little or no effect. No evidence for any requirement for Leu-$3a^+$ (helper/amplifier) cells was obtained in these experiments.

Removal of Suppressor Cells Allows Allogeneic Help. The demonstration of allosuppressor cells in co-culture experiments raises the question of whether their activation, rather than MHC-controlled genetic restriction, may be important in preventing T cell help across an allogeneic barrier. To test this hypothesis, E^+ cells were depleted of Leu-$2a^+$ (suppressor/cytotoxic) cells and cultured with allogeneic or autologous E^- cells and antigen. In the absence of T cell help, cultures of E^- cells made no antibody, showing that the response was T cell dependent. As expected, autologous, but not allogeneic E^+ cells, fully reconsti-

TABLE VI
Phenotype of Allogeneic Suppressor Cells

E^+ Cells added* to PBM	X31 antibody response		
	Exp. 1	Exp. 2	Exp. 3
	ng/ml		
None	45 ± 15	145 ± 12	46 ± 20
Autologous	47 ± 11	ND	46 ± 16
Allogeneic	26 ± 9	1 ± 1	3 ± 1
Allogeneic $2a^{+}$‡	0	4 ± 1	3 ± 1
Allogeneic $2a^-$	71 ± 5	138 ± 21	80 ± 32

* 2×10^5 E^+ cells were added to 4×10^5 PBM in microcultures stimulated with A/X31.
‡ In some experiments, the reciprocal $3a^-$ and $3a^+$ subsets were used instead of $2a^+$ and $2a^-$, with the same result.

TABLE VII
Depletion of Leu-$2a^+$ (Suppressor/Cytotoxic) T Cells Permits Expression of Allogeneic Help

Th cell population*	X31 antibody response		
	Exp. 1	Exp 2	Exp. 3
	ng/ml		
None	2 ± 1	0	3 ± 1
Autologous E^+	283 ± 140	183 ± 72	213 ± 8
Autologous $2a^-$	730 ± 100	114 ± 65	166 ± 10
Allogeneic E^+	11 ± 6	24 ± 7	6 ± 1
Allogeneic $2a^-$	325 ± 118	110 ± 21	202 ± 6
Allogeneic $2a^+$	0	1	0

* One million Th cells were added to cultures of 0.5×10^6 E^- cells and antigen. In some experiments, positively selected $3a^+$ cells were used rather than $2a^-$ populations, with the same result. In each experiment, the E^+ and E^- donors had no HLA-A, -B, -C, or -DR alleles in common.

tuted the antibody response. However, after the removal of Leu-$2a^+$ cells, allogeneic T cells were also able to provide T cell help to responding B (E^-) cells, even when no HLA-A, -B or -DR alleles were shared (Table VII). This experiment was performed many times, always with the same result. The helper activity of allogeneic and autologous Leu-$3a^+$ cells was also compared by titrating different numbers of Th into 1.5×10^5 E^- cells plus antigen. Both allogeneic and autologous $3a^+$ cells were equivalent in their ability to provide help to E^- cells (Fig. 2).

Discussion

Specific antibody responses in cultures of human PBM stimulated with influenza viral antigen were shown to be profoundly suppressed by allogeneic T cells. The human allogeneic Ts had many features in common with those described in murine systems, including relative radiosensitivity (Table I and references 11 and 13), T suppressor/cytotoxic cell phenotype (Table VI and reference 13), and activation by class I MHC antigens (11, 18, 19).[2]

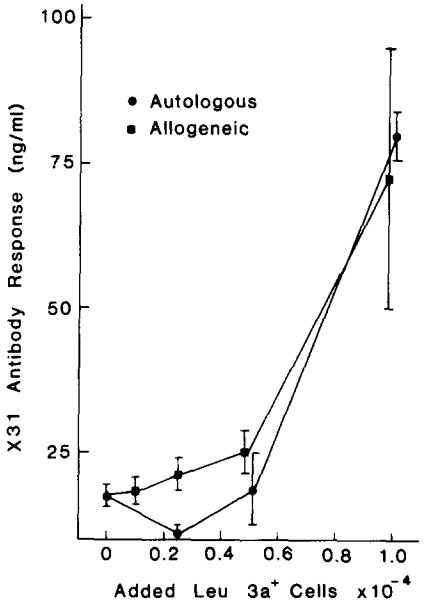

FIGURE 2. Helper activity of autologous (●) and allogeneic (■) Leu-3a$^+$ cells. Positively selected Leu-3a$^+$ cells were prepared by panning from autologous and allogeneic E$^+$ cells and were added in varying numbers to cultures of E$^-$ cells and antigen. Specific antibody production was assayed after 7 d. Comparable T cell help was obtained in both autologous and allogeneic combinations.

The mechanism of allogeneic T cell suppression and its relationship to other forms of regulation is unclear. The possibility that allogeneic Ts may be cytotoxic cells can be reasonably excluded for a number of reasons. First, suppression can be obtained with effector to target ratios of <0.1, which is much lower than required for cytotoxicity (13). Second, no cytotoxicity can be detected in these cells by conventional ^{51}Cr-release assays, and mixed leukocyte culture (MLC)-induced cytotoxic T cells added to antibody cultures do not suppress antibody production (30). Third, conditions that result in allogeneic suppression of secondary IgG antibody responses actually enhance primary responses (30). Finally, cytotoxic T cells require class II-restricted Th cells for optimal activity, yet potent suppression can be obtained with T cells that differ at only a single class I locus, with no evidence of synergy in the case of combined class I and II differences (11).[2]

There are two other explanations for allogeneic suppression that also need to be considered. On the one hand, exposure to alloantigens in the presence of the stimulating antigen may result in the activation of normal, antigen-specific Ts by an abnormal or nonphysiological pathway similar to that described by Bromberg et al. (28) for activation of DTH Ts by allogeneic I-J. Alternatively, suppression could result from quite nonspecific allogeneic effects such as the consumption of essential growth factors by alloactivated T cells (4). Three sets of experiments were designed to distinguish between these alternatives, all of which gave results consistent with the alloactivation of antigen-specific Ts rather than nonspecific allogeneic effects. In the first of these experiments, allogeneic T cell suppression

of in vitro antibody production was compared in cultures stimulated with A/X31 and PWM. Under the same culture conditions, allogeneic T cells profoundly suppressed specific antibody formation, but had little or no effect on PWM-induced polyclonal Ig production (Table II). Next, the frequency of allogeneic Ts in PBM was estimated by limiting dilution analyses in cultures stimulated with A/X31. Because the comparatively low number of replicates (20) used in the limiting dilution assays (due to the problems of obtaining large numbers of PBM from individual donors) may result in high standard deviations in the estimate of Ts, these experiments were repeated several times with different donors. The range of frequencies obtained was 0.8×10^{-5} to 4.5×10^{-5} (mean, $1.9 \times 10^{-5} \pm 0.6$) (Table III). These results are comparable to those obtained in the mouse by Corley et al. (31) for the frequency of alloactivated Ts primed to murine H-2^k or H-2^b MHC antigens (5×10^{-4} to 5×10^{-5}), and are more akin to that expected for antigen-specific Ts than alloreactive cells.

Finally, an experimental approach based on the limiting dilution assays was designed to determine whether the allogeneic T cell suppression was indeed antigen specific. By adding limiting numbers of allogeneic T cells to cultures stimulated with two non–cross-reacting antigens (influenza viruses A/X31 and B/HK), it was possible to segregate suppression of antibody formation to each of them. Thus, in some cultures, the antibody response to A/X31, but not B/HK, was suppressed, and vice versa (Table IV). Moreover, the frequency of wells in which the response to both antigens was suppressed was not significantly different from that predicted from the calculated frequencies of specific allogeneic Ts (Table V). These results show that the Ts, although activated by exposure to allogeneic cells, actually suppress in vitro antibody responses in an antigen-specific manner. In theory, this result could be obtained with either antigen-specific or idiotype-specfic Ts. The latter possibility is supported by a recent report of a human Ts clone that appears to be specific for the antigen receptor on an autologous Th cell clone (32). Our data do not distinguish between these alternatives although it may be possible to do so by depletion of antigen-binding Ts by panning or by antigen-specfic suicide (33).

The demonstrable specificity of allogeneic Ts, whether for antigen or idiotype, lends weight to the argument that these cells play a normal physiological role in regulating human B cell responses, but are activated in an abnormal fashion in culture with allogeneic PBM. Such an interpretation is supported by the recent results of Bromberg et al. (28, 34) who showed that suboptimal doses of antigen on autologous cells given intravenously induces pre-Ts that can then be activated to express suppressor function by interaction with allogeneic cells. Genetic mapping of this effect has identified the allogeneic signal on cells, or in soluble allogeneic effect factor, as arising from differences at the I-J locus. Although this mechanism may not be involved in the generation of allogeneic Ts by class I differences alone as described by Waldmann (11) or Swain (19), it does raise the possibility of Ts induction by a human I-J equivalent in the experiments reported here. (The genetic requirements for alloactivation of Ts are the subject of another report.)[2] Exactly how Ts can be activated by allogeneic signals is, however, unclear. One possible explanation is that a second signal normally provided by Ts inducer cells (35) is supplied either directly by interaction with

alloantigen, or by souble growth factors generated in the allogeneic response. Our inability to demonstrate any requirement for Ts inducer cells in this response (Table 6)[2] supports this interpretation. We have not, however, been able to obtain suppression with SN obtained from these cultures, suggesting that soluble factors are not involved (unpublished observations).

It is interesting to speculate on the physiological reasons for the existence of these cells. The phenomenon of allogeneic suppression can of course be dismissed as an incidental side effect of normal antigen recognition by T cells; for example, by cross-reactivity between antigen X plus MHC A and antigen Y plus MHC B. On the other hand, it is possible that allogeneic interactions of this type are physiologically important. For example, they could conceivably be involved in maternal tolerance to fetal cells (10). Indeed, allosuppressor cells have been demonstrated in a multiparous woman that specifically suppress MLR reactivity to paternal DW alloantigens (9). It is also noteworthy that alloactivated Ts may be responsible in some circumstances for the failure of MHC-incompatible combinations of T and B cells to collaborate in T cell-dependent antibody responses. Thus, Waldman (11) has shown that in vitro antibody responses can be restored in allogeneic (H-21 compatible) combinations of T and B cells by irradiation of the T cells or by using low numbers of T cells to dilute out the allogeneic Ts. Similarly Swain et al. (12) have reported allogeneic T cell help across a total H-2 barrier ($H\text{-}2^d$ T cells and $H\text{-}2^k$ B cells) provided that the suppressor cells are eliminated by treatment with anti-Ly-2 serum and complement or a combination of velocity sedimentation and treatment with mitomycin C. We have previously shown that low doses of irradiation abrogate allogeneic Ts in human E^+ cells and allow collaboration with E^- cells across an MHC barrier, but we did not know whether irradiation of a Th cell may alter its requirements for recognizing antigen in association with self-MHC (14). In the experiments reported here, depletion of Leu-$2a^+$ (suppressor/cytotoxic) T cells by panning or on the FACS allowed T cell help to be expressed across an HLA-A, -B and -DR barrier (Table VII). This experiment was performed several times with different donors, which decreased, but did not exclude the possibility of shared MB or SB loci, either of which may act as MHC-restricting elements (36, 37), and allow effective T-B cell interactions. Moreover, there may be a number of other class II loci expressed on human antigen-presenting cells that have not yet been identified, but which could fulfill this function (38). Nonetheless, it is clear that allogeneic combinations of T and B cells in humans as well as mice may be unable to collaborate in specific antibody responses because of the alloactivation of Ts rather than reasons of MHC restriction. This fact needs to be taken more into consideration in experiments designed to test T cell responses to antigen in association with MHC products.

Summary

Specific antibody responses to influenza virus were obtained in vitro from human blood mononuclear cells (PBM). The addition of allogeneic T cells to responding PBM profoundly suppressed antigen-induced antibody responses, but had no effect on pokeweed mitogen (PWM)-induced polyclonal Ig formation. This raised the possibility that suppression by allogeneic T cells may result from

the activation of antigen-specific T suppressor (Ts) cells rather than nonspecific allogeneic effects. The frequency of allogeneic Ts in PBM from a number of different donors, estimated in a series of limiting dilution analyses, was found to range from 0.8×10^{-5} to 4.5×10^{-5}, which is more typical of antigen-specific than alloreactive T cells. By adding limiting numbers of allogeneic T cells to antibody-forming cultures stimulated simultaneously with two non–cross-reacting antigens (influenza A and B strain viruses A/X31 and B/HK), it was possible to demonstrate suppression of the response to one antigen, but not the other, in the same culture well. Moreover, the frequency of wells in which the response to both antigens was suppressed was not significantly different from that predicted from the calculated frequency of specific allogeneic Ts. These results show that allogeneic suppression was antigen specific, and was not due to nonspecific allogeneic effects. By separating T cell preparations into Leu-3a$^+$ (helper) and Leu-2a$^+$ (suppressor/cytotoxic) T cell subsets, suppression was shown to be mediated by a radiosensitive Leu-2a$^+$ T cell. The removal of Leu-2a$^+$ cells from T cell preparations abrogated the suppressor effect and permitted T cell collaboration with B cells, across an HLA-A, -B, and -DR barrier. This result shows that in at least some combinations, suppression rather than major histocompatibility complex restriction is the reason for the failure of allogeneic T and B cells to collaborate in T cell-dependent antibody responses.

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