Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 889–892, February 1982
Immunology

Microenvironmental immunoregulation: Possible role of contrasuppressor cells in maintaining immune responses in gut-associated lymphoid tissues

(immunosuppression/Peyer's patches)

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ABSTRACT **The addition of Peyer's patch T cells from most strains of mice to spleen cells in primary Mishell–Dutton cultures either has no effect or augments the spleen cells' response to sheep erythrocytes. However, if the Peyer's patch T cells are treated with an anti-I-J antiserum and complement to remove contrasuppressor-inducer cells, the remaining Ly-2 cells (T cells that express Ly-2 but not Ly-1) are highly suppressive. This "latent" suppressor cell activity also can be revealed by removing contrasuppressor-acceptor (transducer) cells from the splenic assay population with either an anti-I-J or anti-Ly-2 antiserum. These findings, taken together with previous work showing that orally administered antigen leads to systemic tolerance, give experimental support to the notion that contrasuppression may be important in allowing microenvironmental immune responses (in this case the gut-associated lymphoid tissue) to take place while systemic immunity is suppressed.**

A novel immunoregulatory T-cell activity, referred to as contrasuppression, has been described (1–4). The contrasuppressor-effector cell can block the activity of both suppressor cells and their cell-free mediators and, in so doing, protect Ly-1 (T cell that expresses Ly-1 but not Ly-2) helper cell activity. Contrasuppressive activity is dependent on the activity of at least three cell types: an Ly-2 (T cell that expresses Ly-2 but not Ly-1) inducer cell, an Ly-1,2 (T cell that expresses both Ly-1 and Ly-2) "transducer" cell,* and an Ly-1 effector cell. These three cells compose a contrasuppressor "circuit" and bear an *I-J* subregion-controlled determinant that is serologically distinct from the I-J determinant found on cells and molecules in the feedback suppressor circuit (ref. 1; unpublished observations).

The potential importance of contrasuppressor activity in *in vivo* immune responses was shown by using the effector cells of the circuit to convert a classical tolerogen into a potent immunogen, without any further manipulation of the experimental animals (4). In addition, the cells of this circuit are very active in the spleens of hyperimmune animals (unpublished observations), which also suggests that contrasuppression may play an important part in *in vivo* physiological function.

Because contrasuppressor cells can protect helper cells from high levels of suppressor-cell activity, we postulated that contrasuppression might allow microenvironmental immune responses to occur in cases where the systemic immune response was highly suppressed (1). We argued that the gut would be an ideal place to look for such microenvironmental regulation. On a teleological basis, it would seem important to maintain high levels of immunity at the surface of the intestinal tissue, where the system must protect itself from large numbers of pathogenic microorganisms. On the other hand, because many nonpathogenic proteins might enter the system through the gut, it would be important for the systemic immune response to be suppressed against these antigens to inhibit allergic or immune-complex complications.

In addition to this teleological argument, there is a body of evidence indicating that antigens entering the system through the portals of the gut are more tolerogenic than when they enter through other portals (see refs. 5 and 6 for review), giving some experimental credence to the above teleological argument. Therefore, we asked the experimental question of whether endogenously activated cells of the contrasuppressor circuit could be found in Peyer's patches. They can. Thus, these results could help in the analysis of what was recently referred to in an editorial in *Lancet* (6) as "the paradox that orally encountered antigen can induce protective immunity and systemic tolerance."

MATERIALS AND METHODS

Animals. Mice were obtained from The Jackson Laboratory or from the breeding facilities at Yale.

Antigens. Sheep erythrocytes (SRBC) were obtained from Colorado Serum, Denver, CO.

Preparation of Peyer's Patch Lymphocyte Populations. Peyer's patches were snipped from the small intestine with sharp curved scissors and placed in phosphate-buffered saline supplemented with 10% (vol/vol) fetal calf serum, 100 mM glutamine, and a mixture of penicillin, streptomycin, gentamycin, and mycostatin. Unfractionated Peyer's patch cells were washed prior to separation by shaking in a 50-ml plastic centrifuge tube. This procedure was repeated three times to remove any adherent bowel contents or debris. Culture contamination was unusual after this procedure. The cells were then ground between the frosted ends of glass slides, and cells were washed three more times before suspension in tissue culture medium.

Cell Fractionation. Anti-Ly-1.2 and anti-Ly-2.2 antisera were supplied by F. W. Shen. They were prepared and tested

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Abbreviations: C, complement; GALT, gut-associated lymphoid tissue; Ly-1 cell, a T cell that expresses Ly-1 but not Ly-2; Ly-1,2 cell, a T cell that expresses both Ly-1 and Ly-2; Ly-2 cell, a T cell that expresses Ly-2 but not Ly-1; PFC, plaque-forming cell(s); SRBC, sheep erythrocytes.

* A transducer cell is one that accepts the inducer cell signal and then helps to generate effector-cell activity either by differentiation into effector cells or by releasing soluble amplifying products, or both.

for specificity as described (7). Congenic anti-I-J^b was provided by D. B. Murphy. Spleen cells (10^7 cells per ml) in appropriately diluted antiserum were incubated at room temperature, centrifuged, resuspended in a 1:5 dilution of rabbit complement (C) (prescreened for low background cytotoxicity), and then incubated at 37°C for 40 min.

T cells were prepared by adding unprimed cells to plastic Petri dishes coated with goat anti-mouse immunoglobulin and harvesting the nonadherent fraction (8).

***In Vitro* Primary Anti-SRBC Response.** A modification of the cell culture technique described by Mishell and Dutton was used to generate *in vitro* primary anti-SRBC responses (1). Spleen cells were suspended in RPMI-1640 tissue culture medium supplemented with 10% fetal calf serum, 100 mM glutamine, 25 mM Hepes, and 50 μM 2-mercaptoethanol. Spleen cells (or the equivalent after antiserum treatment; 1×10^6) were cultured in 0.2 ml with 0.025 ml of a 1% SRBC suspension in Falcon 3040 Microtest II tissue culture plates in a 5% CO_2/95% air incubator at 37°C. At day 4, the anti-SRBC response was determined by enumerating the number of plaque-forming cells (PFC) per culture by the Cunningham technique (9).

RESULTS

Detection of T Suppressor Cells in Peyer's Patches by Removing I-J^+ Cells from the Assay Spleen Cells. We mixed various doses of Peyer's patch T cells with unfractionated spleen cells to look for possible interactions between the two cell populations. Some of the spleen cells were treated with an anti-I-J antiserum and C, which had been shown to remove contrasuppressor cells (1–4). Such an experiment (Table 1) showed that the three doses of Peyer's patch T cells used exerted no inhibitory effect on unfractionated spleen cells. In fact, at one cell dose, (2×10^5), there might even have been a helpful interaction between the two cell populations. In contrast, the same cells added to spleen cells that had been treated with anti-I-J antiserum and C produced a significant suppressive effect, even though this treatment had no effect on the control response (i.e., spleen cells with no Peyer's patch T cells added). Interestingly, the most suppressive effect was found when 2×10^5 Peyer's patch T cells were added to the anti-I-J-treated spleen cells. This was the same dose that produced significant augmentation of the response of the unfractionated spleen cells. If one compares the PFC response in these cultures with the response of cultured spleen cells that did not have Peyer's patch cells added to them, one finds that the anti-I-J-treated spleens made a response that was 25% of this control (75% suppression). However, it might be more appropriate to compare the effect of Peyer's patch cells on unfractionated spleen cells with their effect on anti-I-J-treated spleen cells. This comparison yields a value of 89% suppression. Thus, it is clear that Peyer's patch T cells have quite high levels of potential suppressive activity that can inhibit primary Mishell–Dutton cultures to SRBC. However, these suppressor cells cannot be seen unless at least one cellular member of the contrasuppressor circuit is removed from the cultures. It is also worth noting the unusual dose–response profile. This lack of stoichiometry is always found in these types of experiments, indicating the complex nature of the cell interactions that are taking place in the cultures.

Table 1. Unimmunized Peyer's patch T cells show suppressive activity if an I-J^+ cell is removed from the assay spleen cell population

Peyer's patch T cells, no. $\times 10^{-5}$	Spleen cells*	
	Unfractionated, PFC/culture	Treated with anti-I-J and C, PFC/culture
0	1250 (std.)	1200 (std.)
1	1240 (0)	600 (50)
2	2700 (0)	300 (75)
3	1200 (5)	500 (58)

* 10^6 cells; percentage of suppression of the standard (std.) response is in parentheses.

Detection of Ly-1^-;Ly-2^+ T Suppressor Cells in Peyer's Patches by Removing Ly-2^+ Cells from the Assay Spleen Cells. The transducer cell* in the contrasuppressor circuit expresses Ly-2 alloantigens; therefore, we asked if we could reveal suppressive activity in Peyer's patch T cells by removal of these cells from the splenic assay cultures to which the Peyer's patch cells were added. In addition, we determined the phenotype of the suppressor cell that was uncovered in the Peyer's patch T cell populations. We found (Table 2) that unfractionated spleen cells could not be suppressed by Peyer's patch T cells. (Again, note the peculiar dose–response curve and the augmentation by several doses of Peyer's patch T cells added to unfractionated spleen cells). On the other hand, the same Peyer's patch cells suppressed the anti-Ly-2-treated spleen cells quite profoundly, particularly at the dose of 3×10^5 T cells. Also, the suppressor cells in the Peyer's patch population expressed the Ly-2 alloantigen and not the Ly-1 (see groups B and C in Table 2). Therefore, activated Ly-2 suppressor cells are present in Peyer's patch T cells but only can exhibit their suppressive effect if an I-J^+;Ly-2^+ cell is removed from the spleen cell cultures with which they are interacting.

Detection of Ly-1^-;I-J^- T Suppressor Cells in Peyer's Patches by Removing an Ly-1^-;I-J^+ T Cell from the Peyer's Patch Cells. In the next series of experiments, we asked the question of whether unfractionated spleen cells could be suppressed if an Ly-2 T cell, which expresses the I-J determinant found on contrasuppressor inducer cells (1–4), was removed from the Peyer's patch T-cell populations. Such an experiment (Table 3) showed the inability of unfractionated Peyer's patch T cells to suppress unfractionated spleen cells (group A) and the usual, unusual dose–response profile. Ly-1^- cells from the Peyer's patch T-cell population (group B) were also not suppressive and showed a "helper"-like effect but at a different cell concentration than when unfractionated Peyer's patch T cells were used. Most importantly, the results show that Ly-1^-;I-J^- cells in Peyer's patch T cells can suppress the response of the unfractionated spleen cells (group C). Thus, a cell in the Peyer's patch T-cell population (phenotype: Ly-1^-;I-J^+) is responsible for inducing another cell (phenotype: Ly-1,2;I-J^+) to block the suppressive activity of T suppressor cells (phenotype: Ly-1^-;I-J^-) because removal of the I-J^+ cell from the Peyer's patch T-cell population revealed extremely potent suppressive activity in the remaining T-cell population.

In summary, removal of the contrasuppressor-inducer cell allowed Peyer's patch T cells to suppress nonimmunized unfractionated spleen cells, whereas removal of the contrasuppressor-transducer cell from the spleen cell population allowed unfractionated Peyer's patch T cells to exert potent suppressive activity.

Possible Major Histocompatibility Complex-Determined Control of Peyer's Patch Contrasuppressor Cells. There is an interesting "*Ir* gene-like" effect in this system. Mice that express the *d* haplotype at the *D* locus do not exhibit the contrasuppressive activity we have reported. In 21 experiments out of 24 done, mice with the D^d gene (including F1 mice) failed to show the contrasuppressive activity (i.e., their Peyer's patch cells suppressed unfractionated spleen cells). On the other

Table 2. Ly-1$^-$,2$^+$ T cell in the Peyer's patch suppresses Ly-2$^-$ spleen cells

Peyer's patch T cells added			Spleen cells*	
Group	Treatment	No. × 10^{-5}	Unfractionated, PFC/culture	Treated with anti-Ly-2 and C, PFC/culture
—	—	—	1000 (std.)	3500 (std.)
A	C	1	3600 (0)	1900 (46)
	C	2	960 (2)	1700 (52)
	C	3	2100 (0)	550 (84)
B	Anti-Ly-2 with C	1	4300 (0)	2900 (17)
		2	3200 (0)	2700 (23)
C	Anti-Ly-1 with C	1	1500 (0)	2100 (40)
		2	2700 (0)	1400 (60)

* 10^6 cells; percentage of suppression of the standard (std.) response is in parentheses.

hand, in 18 of 18 experiments done with Peyer's patch T cells from mice expressing a different haplotype at the *D* locus, potent contrasuppression was induced. Particularly revealing were the differences between the B10.A(2R) and the B10.A strains that differ from each other only at the *D* region of the major histocompatibility complex. The B10.A(2R) (D^b) mice showed contrasuppression, whereas the B10.A (D^d) strain did not.

DISCUSSION

Ly-2 T cells from Peyer's patches of mice that have not been immunized with SRBC can suppress *in vitro* spleen cell responses to SRBC if the cell circuit responsible for contrasuppression is quenched, either by removing the contrasuppressor-induced cell with an I-J reagent or by eliminating the acceptor (transducer)* cell with either an Ly-2 or an anti-I-J reagent. Thus, contrasuppressor-inducer cells localized in Peyer's patches interact with contrasuppressor-transducer cells from the spleen to block the appearance of suppression.

The presence of both activated suppressor-effector and contrasuppressor-inducer cells in Peyer's patches suggests a role for contrasuppression in immune responses that take place in local microenvironments. Suppressor cells are likely to be generated by antigens entering the gut-associated lymphoid tissue (GALT), but local immunity can be maintained by induction of the contrasuppressor circuit. If the local suppressor cells (and not the contrasuppressor cells) subsequently migrate to the spleen (10), systemic immunity would be suppressed and the teleological scenario put forth in the introduction would be fulfilled. However, the antigen used in these studies, SRBC, is a good systemic immunogen; thus, two *ad hoc* assumptions must be made to make the experimental results compatible with the theory.

One must first address the question of why there are such potent suppressor cells of the anti-SRBC response in the GALT. Perhaps there are crossreacting antigens, heterophile in nature, that activate the GALT, or perhaps the suppressor cells are polyclonally or nonspecifically activated. This being the case, one must explain why these suppressor cells fail to inhibit systemic immunity to SRBC. One could postulate that the ratio of systemic suppressor cells to helper cells is lower in the *in vivo* system than in the *in vitro* cultures we used. There is good evidence that if mice are fed SRBC, their systemic immune response is suppressed (10, 11) and generally requires several feedings for suppression to be seen (11). Thus, the findings are not necessarily at odds with the hypothesis, and potential contradictions could be explained by quantitative factors.

Our failure to demonstrate contrasuppressor cells in the GALT of mice that express the D^d haplotype is potentially interesting. It is possible that this is an "*Ir* gene" defect in the cells of the contrasuppressor circuit. If this turns out to be true, it would be further evidence separating the contrasuppressor system from the helper system because the *Ir* genes responsible for immunodeficiencies in the two systems would map to different regions of the major histocompatibility complex. This possibility is made less remote by the recent finding that splenic dendritic cells, which are extremely efficient in inducing immune responses that are resistant to suppressor cells (unpublished data), express high levels of both *H-2D* and *I-A* gene products (12). This could give us important clues to the questions of how or why the contrasuppressor circuit is turned on. It also should allow us to test the importance of contrasuppressor cells in GALT by seeing if mice that express $H\text{-}2D^d$ are particularly sensitive to certain microbial pathogens, particularly those that induce antibodies that crossreact with SRBC.

Table 3. Removal of an I-J$^+$ T cell from the Peyer's patch cells allows suppression to be seen without need for treatment of the assay spleen cells

Peyer's patch T cells added			Unfractionated spleen cells,*
Group	Treatment	No. × 10^{-5}	PFC/culture
—	—	—	1250 (std.)
A	None	1	1240 (0)
	None	2	2700 (0)
	None	3	1200 (5)
B	Anti-Ly-1 + C	1	1600 (0)
		2	1000 (20)
		3	3640 (0)
C	Anti-Ly-1 +	1	250 (80)
	anti-I-J + C	2	400 (68)
		3	350 (72)

* 10^6 cells; percentage of suppression of the standard (std.) response is in parentheses.

Some mice were provided from a Yale University breeding facility supported by Central Resource Facility Grant CA-16359. This work was supported in part by Grants CA-08593, CA-14216, and CA-29606. D.R.G. was supported by Training Grant AI-07019.

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