Brief Definitive Reports

REGULATION OF ANTIBODY RESPONSE BY CELLS EXPRESSING HISTAMINE RECEPTORS*

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The in vivo immune response is subject to many modifying factors, both cellular and humoral (1–8). Modifiers of immunity may act either as enhancers or suppressors. One of the most widely studied examples of cellular immunological enhancement is the cooperative effect demonstrated between thymus- and marrow-derived cells for humoral antibody production (1, 2). Cell synergism for amplification of graft-*versus*-host reaction has also been reported (3). In contrast, suppression of humoral antibody responses has been obtained using thymus-derived cells (4). Humoral factors such as antibody (see reference 5 for review) and other diffusable cell products can also exert suppresive (6) or augmenting (7–10) influences on immunity.

Affinity column chromatography of immunocompetent cells has been achieved by attaching antigenic molecules to glass, plastic, polyacrylamide, or agarose beads (11–13) or to nylon threads (14). It was demonstrated that cells expressing antibody-like receptors on their surfaces were retained by the columns, whereas cells not possessing the relevant recognition receptors passed through the columns.

Recent studies have indicated that histamine, beta catecholamines, and prostaglandins E_1 and E_2 can inhibit both the cytotoxic effects of lymphocytes sensitized to fibroblasts or mast cells (15) and the IgE-mediated release of histamine from human leukocytes (16). Furthermore, receptors for histamine have been demonstrated on human peripheral blood leukocytes and mouse spleen cells (17). Histamine has been insolubilized by chemical linkage via a protein carrier to agarose (Sepharose) beads (17–18). Cells with preformed histamine receptors on their surfaces were specifically bound to the histamine-protein-Sepharose beads.

Since the technical aspects of cell chromatography and the attachment of histamine to Sepharose beads are known, and previous studies imply that

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histamine may serve as a modifier of immune processes (15, 16), it was of interest to determine whether spleen cells passed over histamine-rabbit serum albumin-Sepharose (H-RSA-S) columns would exhibit an immune response potential different from that of unpassed cells or of cells passed over rabbit serum albumin-Sepharose (RSA-S) control columns.

Materials and Methods

Preparation of H-RSA-S and RSA-S Beads.—Histamine was conjugated to rabbit serum albumin (RSA) by incubating a solution of histamine with 1-ethyl-3-(3 dimethylaminopropyl) carbodimide (17). The resulting histamine-rabbit serum albumin conjugate (H-RSA) or RSA alone was attached to Sepharose 4 B (Pharmacia, Uppsala, Sweden) activated by CNBr.

Preparation of Cell Suspensions and Filtration over H-RSA-S and RSA-S Columns.—Spleen cell suspensions were prepared in Eagle's medium (Microbiological Associates, Inc., Jerusalem, Israel) as described elsewhere (19). The concentrations were estimated by repeated sample counting using a hemacytometer.

Aliquots of the cells (2.5×10^7) to be filtered over the H-RSA-S or RSA-S columns were incubated at 37°C for 15 min with 0.6 ml of a 25% suspension of either H-RSA-S or RSA-S in Eagle's medium. The cell-bead mixtures were then poured into columns (made from plastic pipettes and containing a small piece of sponge in the bottom so as to retain the Sepharose but to permit passage of unbound cells), and the unattached cells were collected after the column had been washed twice with 1 ml of Eagle's medium. The cells that passed through the column were then recounted and adjusted to the concentration desired for injection.

Cell Transfers and PFC Assays.—(BALB/c × C57BL/6)F_1 female mice, 9–12 wk of age, were used as donors and recipients. Recipients were exposed to 750 rads of ^{60}Co gamma irradiation and injected via the tail vein with 5×10^6 spleen cells mixed with 3×10^8 sheep erythrocytes (SRBC) per recipient in a total volume of 1.0 ml. The recipient spleens were removed either 7 or 13 days after transfer and their contents were individually assayed for direct and indirect plaque-forming cells (PFC), respectively, using a modification of the hemolytic PFC assay (19) in agarose.

RESULTS

In order to determine whether passage of spleen cells over histamine-coated columns altered the immunocompetent properties of the cells not retained by the columns, and if so whether such an alteration could be attributed to cells bearing histamine receptors on their surfaces, comparisons were made of the direct PFC responses generated by the following cell transfers mixed with 3×10^8 SRBC: (*a*) 5×10^6 spleen cells not passed over columns; (*b*) 5×10^6 spleen cells passed over RSA-S control columns; and (*c*) 5×10^6 spleen cells passed over H-RSA-S columns. The spleen cell suspensions used in the first experiments were prepared from donors which had been immunized with 3×10^8 SRBC 1 wk earlier, since the hemolytic plaque-forming cells of mice immunized with SRBC were shown to express receptors for histamine (18). The results of the direct PFC responses, assayed 7 days after transfer (Table I), indicate that the immunocompetence of spleen cells passed over H-RSA-S columns was enhanced from 2.2- to 5.5-fold when compared with the response generated by an equal number of unfractionated cells, and from 1.6- to 4.4-fold

when compared with responses generated by cells passed over RSA-S control columns. Recipient spleens of the H-RSA-S and unfractionated groups were also assayed 13 days after cell transfer for indirect PFC responses. Inocula of 5×10^6 unfractionated cells generated 23,100 ± 4950 and 3950 ± 580 indirect PFC,

TABLE I

*Direct PFC Responses in Irradiated Mice Injected with 5×10^6 Unfiltered Spleen Cells (No col), 5×10^6 Spleen Cells Passed over RSA-Sepharose Columns (RSA-S), or 5×10^6 Spleen Cells Passed over H-RSA-Sepharose Columns (H-RSA-S) from Immunized Donors**

Experiment No.	Mean No. of direct PFC/spleen			Ratio of direct PFC/spleen		
	No col	RSA-S	H-RSA-S	RSA-S / No col	H-RSA-S / RSA-S	H-RSA-S / No col
1	1060 ± 204	1310 ± 224	4830 ± 873	1.2	3.7	4.6
2	3550 ± 910	4370 ± 793	19,300 ± 2470	1.2	4.4	5.4
3	2370 ± 820	4340 ± 610	6940 ± 1680	1.8	1.6	2.9
4	1960 ± 836	1890 ± 302	4320 ± 680	0.97	2.3	2.2
5	1350 ± 302	1870 ± 844	3040 ± 690	1.4	1.6	2.3
6	1720 ± 494	N.T.	9460 ± 1440	—	—	5.5
7	1830 ± 164	N.T.	7160 ± 586	—	—	3.9
8	1220 ± 292	N.T.	4050 ± 1110	—	—	3.3
9	720 ± 158	N.T.	3490 ± 955	—	—	4.8
10	1350 ± 276	N.T.	3850 ± 1370	—	—	2.9

No col = not passed over columns.

± Indicates standard errors.

N.T., not tested.

* (BALB/c × C57BL/6) F_1 mice exposed to 750 rads of ^{60}Co gamma irradiation were injected with 5×10^6 spleen cells (mixed with 3×10^8 SRBC) from syngeneic donors immunized 7 days before with SRBC. The donor spleen cells were not passed over columns or were passed over either RSA-Sepharose or H-RSA-Sepharose columns before transfer into recipients. Direct PFC assays were made 7 days after cell transfer.

TABLE II

*Direct PFC Responses in Irradiated Mice Injected with 5 or 10×10^6 Unfiltered Spleen Cells (No col), or with 5 or 10×10^6 Spleen Cells Passed over H-RSA-Sepharose Columns (H-RSA-S) from Unimmunized Donors**

Experiment No.	Mean No. of direct PFC/spleen		Ratio of direct PFC/spleen
	No col	H-RSA-S	H-RSA-S / No col
1	537 ± 411	1810 ± 486	3.4
2	188 ± 71	755 ± 38	4.0
3	109 ± 29	1380 ± 342	12.5
4	270 ± 74	1190 ± 640	4.4
5	1860 ± 122	5370 ± 2380	2.9
6	711 ± 195	2620 ± 195	3.7

± Indicates standard errors.

* (BALB/c × C57BL/6)F_1 mice exposed to 750 rads of ^{60}Co gamma irradiation were injected with 5×10^6 (experiments 1–4) or with 10×10^6 (experiments 5 and 6) (mixed with 3×10^8 SRBC) from unimmunized syngeneic donors. The donor spleen cells were not passed over columns or were passed over H-RSA-Sepharose columns before transfer into recipients. Direct PFC assays were made 7 days after cell transfer.

respectively. The above results obtained for direct and indirect PFC suggest that the histamine columns retained cells which act to suppress the antibody response.

Since plaque-forming cells are retained by H-RSA-S columns (18), the enhanced responses observed as a function of cell fractionation could have been due to the removal of antibody-producing cells which might have regulated the level of responsiveness via antibody-feedback mechanisms (5). In order to exclude this possibility, spleen cells from immunized donors were passed over H-RSA-S columns, and equal numbers of the cells not retained by the columns, as well as unfiltered cells, were injected into groups of irradiated recipients with 3×10^8 SRBC. The mean numbers of direct PFC obtained are shown in Table II. An enhancement of from 2.9- to 12.5-fold was obtained in the number of direct PFC generated by the cells passed over H-RSA-S. These results verify those shown in Table I and support the hypothesis that a cell type expressing histamine receptors on its surface has a regulatory effect on the PFC response to SRBC.

DISCUSSION

A few reports have appeared recently assigning to certain cells the capacity to decrease antibody responses (mentioned in reference 4). Although the suppressor function has been ascribed to a population of thymus-derived cells (4), such cells have not been previously associated with a receptor to a physiological substance. The results presented here demonstrate that removal of cells based on a specific interaction of a physiologically active agent, histamine, with its natural receptor can lead to significantly enhanced antibody responses of both the IgM and IgG classes. The fact that H-RSA-S fractionation of spleen cells from unimmunized donors also generated elevated PFC responses indicated that cells possessing regulatory function exist in the splenic compartment before active immunization. Whether these cells are of thymic origin and similar to those described by Gershon et al. (4) has not yet been established.

It is likely that the differences observed in the immune potential of the fractionated and control cells are the result of cell separation by the H-RSA-S column, and are not due to pharmacological effects of histamine on the cells. The drug-Sepharose conjugate does not stimulate cyclic AMP, which would be expected were the pharmacological effects of histamine directly involved (20).

We have previously observed that among the cells possessing histamine receptors are the direct and indirect PFC, which are specifically bound to the histamine-coated columns (18). On the other hand, the present report demonstrates the enhancement of immune response potential upon passing spleen cells over the histamine-coated columns. Retention of PFC by H-RSA-S columns cannot account for the enhanced responses via removal of the source of antibody-feedback suppression, since enhancement of the PFC response was demonstrated using spleen cells from unimmunized donors. Furthermore, a mouse strain has been identified whose PFC are not retained by H-RSA-S

columns, despite the fact that enhanced PFC responses are generated by spleen cell transfers in this strain after passage of splenic precursor cells over H-RSA-S columns (manuscript in preparation). Thus, enhancement of secondary immune response potential was obtained without the concomitant removal of antibody-forming cells.

The results reported here open for investigation a number of questions concerning the nature of this regulatory phenomenon: (*a*) Can the cells bound to H-RSA-S generate an antibody response independently of the cells passed through the column? (*b*) Can a distinction be made between a direct cell-mediated modification of the immune response and a humoral substance which these cells may release? (*c*) Are the cells with regulatory function qualitatively or quantitatively altered by immunization? (*d*) Will addition of the regulator cells back to the passed cells or to a normal suspension of immunocompetent cells suppress responsiveness? (*e*) Does the regulatory cell population constitute a heretofore functionally unidentified class of cells, or is it a subpopulation found in the thymus- or marrow-derived cell compartments? Studies concerned with identification of the source of these cells, and characterization of the cellular and/or molecular events involved in this regulatory process are in progress.

SUMMARY

Spleen cells from immunized and unimmunized mice were either passed over histamine-rabbit serum albumin-Sepharose columns or rabbit serum albumin-Sepharose control columns. The immune response potential of 5×10^6 cells excluded from the two columns were compared with each other, and with an equal number of unfiltered cells by injection of the cell suspensions mixed with sheep erythrocytes into irradiated, syngeneic recipients. The direct and indirect anti-sheep erythrocyte plaque-forming cell responses generated by the cells passed over the histamine-bead column were significantly greater than the responses resulting from the inocula of unfiltered cells or cells passed over control columns. These results indicate the existence of a cell population expressing surface receptors for histamine, which functions to regulate antibody responses.

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