ROSETTE-FORMING ABILITY OF THYMUS-DERIVED LYMPHOCYTES IN HUMORAL AND CELL-MEDIATED IMMUNITY

II. Helper Cell Activity*

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The immune specificity of precursors of antibody-forming cells is mediated by specific immunoglobulin receptors which bind antigen (1, 2). However, the antigen-binding characteristics of T cells are difficult to demonstrate. Although incubation with lethally-radioactive soluble antigen destroys antigen-specific T-cell function (3, 4), helper T cells do not adhere to antigen-specific immunoadsorbents (5).

We previously reported that helper cells in nonimmune spleen do not bind detectable numbers of sheep erythrocytes (SRBC) to form rosettes (6). The present study confirms this observation and presents evidence that helper cells from immune animals also do not form rosettes. In contrast, medium lymphocyte effector cells in cell-mediated immune reactions against SRBC were shown to be T rosette-forming cells (RFC)[1] (7).

Materials and Methods

Details concerning medium, sodium azide and neuraminidase treatment, antigens and immunization, preparation of cell suspensions and formation of rosettes, and velocity sedimentation are published elsewhere (7).

Animals. Male C57BL/6 mice (Canadian Breeding Laboratories, Montreal, Quebec) 6–8 wk of age were used.

Assay for Helper Cells and Precursors of Plaque-Forming Cells (PPFC). Helper cells were assayed by an adoptive transfer system in which each cell fraction was injected with 2×10^7 bone marrow cells and 2×10^8 sheep erythrocytes (SRBC) into lethally irradiated (750 R) syngeneic

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[1] *Abbreviations used in this paper:* PFC, plaque-forming cell; PPFC, precursor of plaque-forming cell; RFC, rosette-forming cell.

recipients. 8 days later, the spleens were removed and assayed for 19S and 7S plaque-forming cells (PFC). Helper cell activity was calculated by the following formula:

$$\text{Activity} = [(A - B) \div C] \times 100\%;$$

where A is the PFC response of animals injected with immune cells plus bone marrow cells, B is the response of animals injected with immune cells alone, and C is the maximum response produced by 2×10^7 bone marrow cells in each experiment.

PPFC were assayed by injecting each fraction with 2.5×10^7 thymocytes plus 2×10^8 SRBC into lethally irradiated recipients. The number of splenic PFC after 8 days in animals injected with a fraction plus thymocytes was proportional to the number of PPFC in the original fraction.

Assay for 19S and 7S PFC. 19S PFC were assayed by the method of Cunningham and Szenberg (8). 7S PFC were assayed by a modification of the method of Pierce et al. (9). Cells were preincubated in one drop of 1/16 dilution of anti-IgM serum (Melloy) at a concentration of 5×10^6 nucleated cells/ml at 25°C for 15 min. This step inhibited 19S PFC without altering the expression of 7S PFC (Fig. 1). Without further washing, one drop of 1/40 dilution of rabbit antimouse immunoglobulin-developing serum (a gift from Dr. H. Pross) was added. The cell mixture was then incubated at 37°C with SRBC and guinea pig complement (C) in a slide chamber. After 90 min, 7S PFC per chamber were counted directly. This method was particularly sensitive for detecting low numbers of 7S PFC.

Results

Rosette-Forming Ability of Helper Cells. Normal spleen cells were subjected to rosette formation, separated into nonrosette (A), T rosette (C and D), and B rosette (E, F, and G) fractions, and were tested for helper cell and PPFC activity. (Fig. 2). Fractions transferred alone produced no significant PFC response.

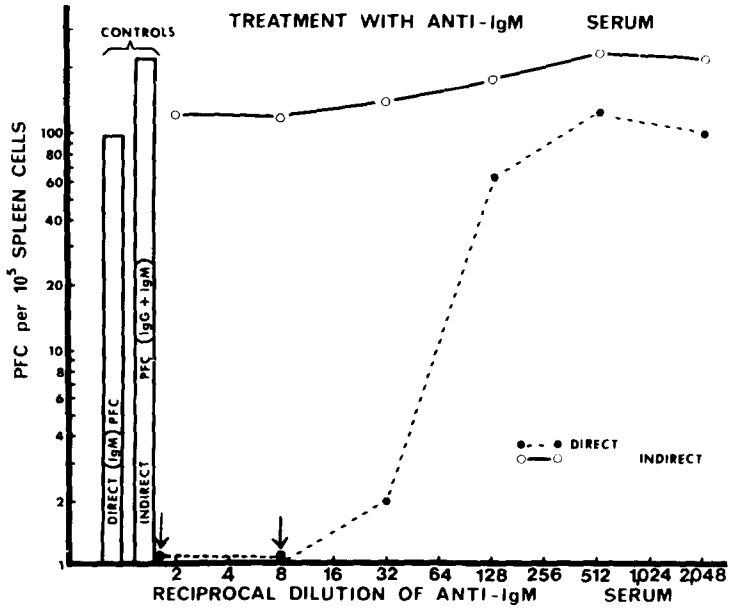

FIG. 1. Spleen cells from 7-day SRBC-immune mice were incubated with different dilutions of anti-IgM serum and assayed for direct (IgM) and indirect (IgM + IgG) PFC. Controls consisted of cells incubated without antiserum.

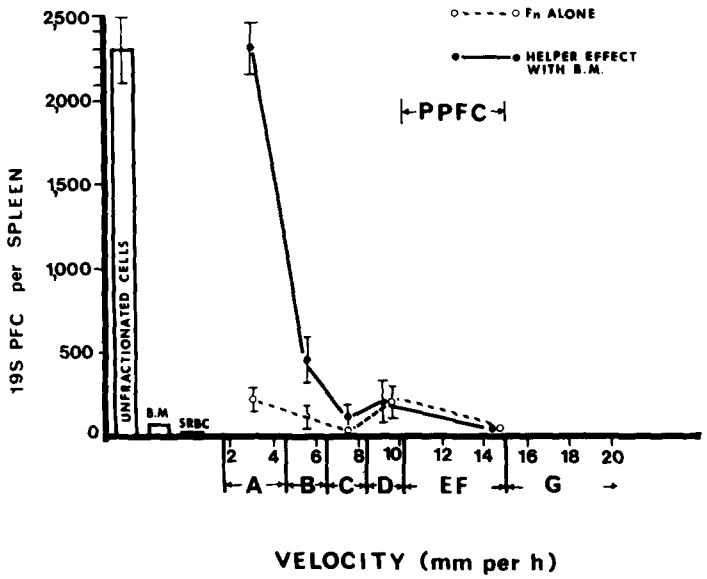

FIG. 2. Separation of RFC by velocity sedimentation. Normal spleen cells were subjected to rosette formation with neuraminidase-treated SRBC and were sedimented in the presence of sodium azide. Each fraction was transferred alone or with bone marrow cells (B.M.). The velocity range of PPFC (80% of the response of unfractionated cells) is indicated. Each point represents the mean ± the standard deviation.

Fractions E and F synergized with thymocytes; PPFC had sedimented as B rosettes binding 10–18 SRBC.

Fraction A synergized with bone marrow cells; helper cells had sedimented as nonrosette-forming small lymphocytes. (The PFC response of fraction A represented 80% of that of unfractionated cells.) Helper cells from nonimmune lymph nodes were also nonrosette-forming lymphocytes (unpublished result). These results confirm in a different mouse strain with a higher adoptive response, our previous report (6).

When immune lymph node cells were fractionated without prior rosette formation, fractions A and B produced significant PFC numbers when transferred alone. When fractions were transferred with bone marrow cells, the number of 19S PFC produced by fraction B increased to 11,000; there was no significant increase in PFC produced by the other fractions. Therefore, the majority of 19S helper cells sedimented in fraction B. (Fig. 3 A).

When rosettes were made from immune cells before sedimenting in the presence of azide, rosettes sedimented in fractions C to G. Most 19S and 7S PFC formed rosettes which sedimented greater than 10 mm/h (unpublished results); 19S PPFC which synergized with thymus cells sedimented as rosettes between 9.5 and 14 mm/h (Fig. 3 B). Rosette formation did not inhibit the activity of unfractionated cells injected with bone marrow cells (Figs. 3 and 4).

When fractions were injected with bone marrow cells, the number of 19S PFC produced by fraction B was 14,000; no significant PFC response occurred in rosette fractions (D to G). 19S helper cells were again associated with fraction B.

Rosette formation therefore had no effect on the sedimentation velocity of 19S helper cells.

Similar results were obtained for 7S helper cells. When cells were sedimented without prior rosette formation, the 7S PFC response with bone marrow was produced by fraction A; no PFC response occurred in the other fractions (Fig. 4 A). When rosettes were made before sedimenting, the 7S PFC response with bone marrow remained in fraction A (Fig. 4 B).

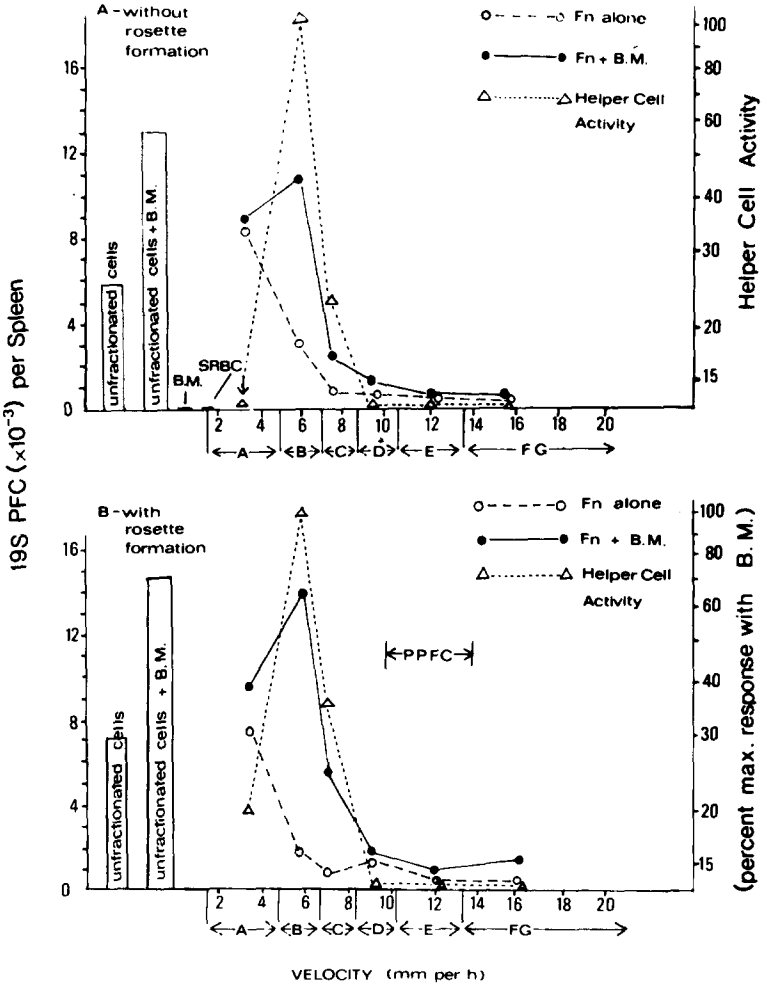

FIG. 3. Effect of velocity sedimentation on 19S helper cells in immune lymph node. (A) Immune lymph node cells were subjected to velocity sedimentation without rosette formation. Each fraction was transferred either with or without bone marrow cells (B.M.). Controls consisted of an equivalent number of unfractionated cells transferred either with or without bone marrow cells, bone marrow cells alone, or SRBC alone. (B) Immune lymph node cells were subjected to rosette formation before sedimenting with sodium azide present. Each fraction was transferred as in A. Each point represents the average of five mice. Helper cell activity in each fraction was expressed as a percent of the maximum response with bone marrow.

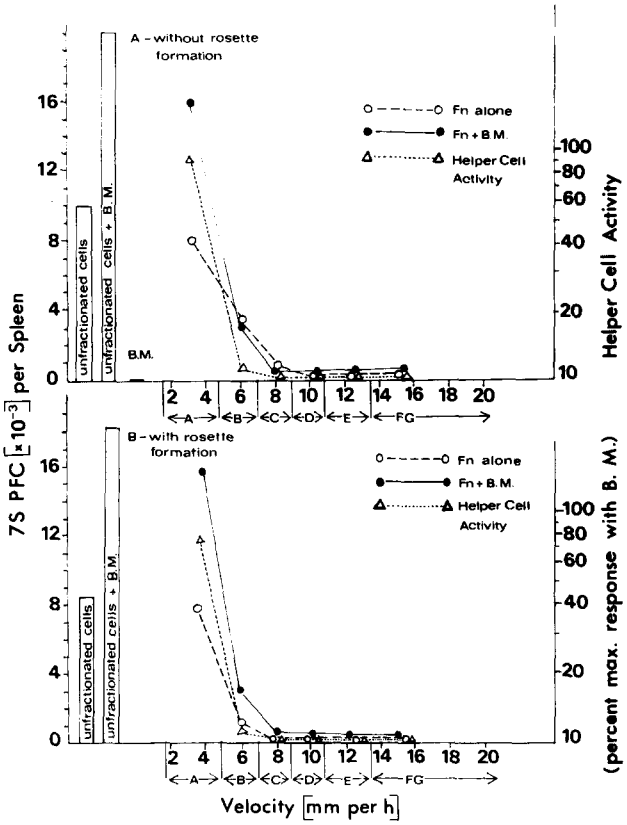

FIG. 4. Effect of velocity sedimentation on 7S helper cells in immune lymph node. Each experimental group from Fig. 3 was assayed for 7S PFC. Helper cell activity in each fraction was determined as in Fig. 3. (A) Without rosette formation and (B) With prior rosette formation.

7S and 19S helper cell activity was associated with nonrosette-forming small and medium lymphocytes, respectively. Small and medium lymphocyte 7S and 19S helper cells observed in the present study may represent different stages of differentiation.

Effect of Immune Cell Dose on Helper Cell Activity. For estimating the relative number of helper cells in each fraction, the dose-response relationship of helper cells was determined in the present system.

Between 5×10^4 and 3×10^5 cells, helper activity was directly proportional to immune cell dose (Fig. 5). At immune cell doses greater than 3×10^5, helper activity no longer increased linearly; instead the PFC response with bone marrow approached a maximum value. PFC values greater than one-third the maximum response may underestimate the helper activity present. Therefore, the PFC responses observed when fraction B (Fig. 3) or fraction A (Fig. 4) was transferred with bone marrow cells may underestimate the helper cell activity in these fractions.

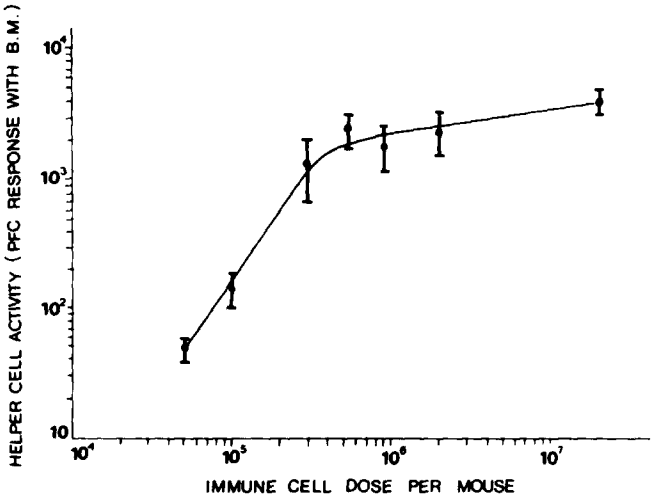

FIG. 5. Effect of immune cell dose on 19S helper cell activity. Different doses of immune cells were transferred either with or without bone marrow cells. Helper activity was the difference between the PFC response of immune cells transferred with bone marrow cells and the PFC response of immune cells alone. Each point represents the mean ± the standard error of five mice. The response of 3×10^6 spleen cells was 33% of the maximum response with bone marrow (4,000 PFC per spleen).

Discussion

The present finding, that helper cells in normal and immune animals do not form rosettes, is in accordance with the work of Wilson (10) who demonstrated that T memory cells do not form rosettes. Furthermore, Roelants and Rydén (11) did not observe any increase in antigen binding to T cells after immunization. Rubin and Wigzell (5) failed to demonstrate specific adsorption of helper cells on antigen-coated Sepharose beads, or significant differences in antigen requirements of T lymphocytes in vitro after immunization.

The failure to demonstrate rosette-forming helper cells does not imply that these cells do not recognize antigen specifically. Indeed, a high degree of specificity of helper cell activity has been demonstrated for some antigens (12, 13). Although SRBC-primed helper cells do not form rosettes, carrier-primed helper cells have been reported to specifically bind carrier-coated erythrocytes (S. Kontianien and J. L. Andersson, personal communication). Helper cells may therefore have a lower affinity for antigenic determinants on erythrocytes, or the determinants recognized by the helper cells may be less accessible on intact erythrocytes and may become exposed only after breakdown of the erythrocyte.

It is unlikely that activated helper cells failed to function within 3 or 4 days after in vivo transfer and were not detected in the 7-day adoptive transfer system. Even under in vitro conditions in which a 19S PFC response developed within 3 or 4 days, fractionated T rosettes failed to synergize with splenic B rosettes from animals immunized with a low dose of SRBC. PFC were produced only when the nonrosette fraction was cultured with splenic B rosettes (unpublished result).

Both in vivo and in vitro approaches indicated that helper cells were nonrosette-forming lymphocytes.

Helper cells described in the present work were distinct from medium lymphocyte effector cells in cell-mediated immunity. Helper cells did not bind SRBC in a manner detected by the rosette assay used. However, under the same conditions of rosette formation, medium lymphocyte effector cells in cell-mediated immunity against SRBC were observed to form rosettes (7). Similarly, Dennert and Lennox (14) have demonstrated that helper cells and cytotoxic T cells primed to a DBA/2 mastocytoma are distinct subsets of T cells. Furthermore, it has been shown that helper cells express Ly-1 whereas cytotoxic cells express Ly-2 surface antigens (Peter Beverley, personal communication). Whether these two cell types represent different cell lineages or different stages of differentiation within the same cell line has not been established.

Summary

The technique of velocity sedimentation at unit gravity was used to determine the size and rosette-forming ability of helper cells in nonimmune and immune C57BL/6 mice. Helper T cells were assayed by the ability to cooperate with bone marrow (B) cells in the antibody response to sheep erythrocytes (SRBC) in vivo. 19S helper cells in nonimmune animals were nonrosette-forming small lymphocytes; after immunization with SRBC in complete Freund's adjuvant, 19S helper cells were nonrosette-forming medium lymphocytes. 7S helper cells in immune animals were small lymphocytes which did not form rosettes. No SRBC binding by helper cells was observed under the conditions of rosette formation used.

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