REQUIREMENT FOR T CELLS IN THE PRODUCTION OF MIGRATION INHIBITORY FACTOR

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The recent demonstrations that migration inhibitory factor (MIF) (1–4), chemotactic factor (5), mitogenic factor (6, 7), and lymphotoxin (8) can be produced by B as well as T lymphocytes have raised serious doubts about the validity of in vitro assays for cell-mediated immunity as indices of T-cell function. Yet there are a number of human disease states in which the biological significance of the in vitro production of mediators by B cells may be called into question. For example, lepromatous leprosy and chronic mucocutaneous candidiasis are characterized by specific functional T-cell deficiency and failure of macrophage killing of ingested organisms despite high levels of circulating antibodies to the offending parasite. If B cells are capable of producing the mediators of delayed-type hypersensitivity, why are they ineffective at initiating macrophage activation in these diseases?

We have pursued the question whether B cells can be activated by antigen to produce MIF in a model that excludes participation of T cells by virtue of an immune response (Ir) gene restriction. Strain 2 guinea pigs respond to immunization with the 2,4-dinitrophenyl (DNP) derivative of poly-L-lysine (PLL) with the production of antibody and delayed-type hypersensitivity; strain 13 animals are nonresponders (9). However, strain 13 animals can respond to DNP-PLL when the molecule is complexed to an immunogenic carrier, such as ovalbumin (10). In this situation, anti-DNP antibodies are produced, but delayed-type cutaneous reactivity and blastogenesis to DNP-PLL are absent (11), indicating an absence of T cells capable of responding to DNP-PLL. This system allows us to test directly the question whether MIF can be produced by interaction of antigen with primed B cells in the absence of competent T cells.

Materials and Methods

PLL hydrobromide (mol wt 90,000) was obtained from New England Nuclear, Boston, Mass. Guinea pig albumin (GPA) was prepared from strain 13 guinea pig serum by the method of Schwert (12). DNP&subscript;32-PLL and DNP&subscript;37-GPA were prepared as previously described (13). The subscripts refer to the average number of DNP groups per molecule. Twice recrystallized hen ovalbumin (Ova) was obtained from Miles Laboratories Inc., Elkhart, Ind.

Preparation of DNP-PLL-Ova Complexes. Equimolar amounts of Ova were added to DNP-PLL in 0.075 M NaCl dropwise with the formation of a precipitate.

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Anti-DNP antibodies were measured by the hapten-binding assay of Stupp et al. (14).

Strain 2 and 13 guinea pigs, 500–600 g, from the National Institutes of Health breeding colony were sensitized with a complex of DNP-PLL-Ova (100 μg each) in Freund's complete adjuvant (H₃7Ra) administered in a total of 0.4 ml distributed over four foot pads. The animals were skin tested with 10 μg/0.1 ml between day 14 and day 21 to confirm their responder/nonresponder status, and animals were used within 14 days of skin testing.

To maximize the number of B cells available, spleens were used in all experiments. The spleen cells were isolated by gentle homogenization in a glass homogenizer and washed three times. They were cultured in the presence or absence of antigen at a cell density of 15 × 10⁶ cells/ml in 1.5 ml per Leighton tube for 24 h in an atmosphere of 7% CO₂ in air at 37°C. The medium was Eagle's minimal essential medium (MEM) containing 5% guinea pig serum. The supernates were harvested and either used directly or quick frozen. Before testing they were adjusted to 15% in guinea pig serum and tested neat and following 1:3 dilution. Migrating cells were 48-h oil-induced peritoneal cells of Hartley strain guinea pigs. In five of six experiments controls consisted of supernates from spleen cells cultured in medium alone which were reconstituted with the appropriate dose of stimulating antigen. Percent inhibition was given by the formula:

\[
\% \text{MI} = 1 - \frac{\text{migration area of supernates of antigen-stimulated cultures}}{\text{area of control supernates reconstituted with antigen}} \times 100.
\]

Because there was some indication that supernates containing reconstituted antigen were somewhat more toxic than those containing antigen cultured with cells in one experiment comparisons were made between spleen cells of sensitized and normal guinea pigs cultured with the antigen. In this experiment, migration inhibition was calculated by the following formula:

\[
\% \text{MI} = 1 - \frac{\text{migration in supernates of sensitized cells plus antigen}}{\text{migration in supernates of nonsensitized cells plus antigen}} \times 100.
\]

The virus plaque assay was carried out on lymph node cells from the same donors using vesicular stomatitis virus as the probe described previously (15).

Results and Discussion

Strain 2 and strain 13 guinea pigs immunized with DNP-PLL complexed to ovalbumin produced essentially equal amounts of anti-DNP antibodies (Table I). Spleen cells from donors of both strains produced MIF in vitro upon culture with the immunizing complex, DNP-PLL-Ova (Table II). In contrast, however, only strain 2 guinea pig spleen cells were capable of being activated by DNP-PLL to produce significant amounts of MIF (Table II).

When the virus plaque assay which enumerates activated T cells (21) was applied to the lymph node cells from the same donors, virus-plaque-forming cells (V-PFC) were stimulated by DNP-PLL-Ova in both responder and nonresponder strains (Table III). However, strain 13 lymphocytes could not be stimulated by DNP-PLL, while strain 2 cells produced 2.4 V-PFC/10³ above background, thus confirming the validity of the virus plaque assay as a useful index of T-cell reactivity.

The simplest interpretation to be drawn from these data would be that B cells interacting with antigen in the absence of functional T cells are incapable of producing MIF, and that antigen-induced MIF production is a T-cell dependent response. Because it is clear that both B cells and T cells are capable of MIF production, the MIF assay may be useful qualitatively to indicate T-cell activation, but the degree of migration inhibition cannot be regarded as a quantitative reflection of the number of sensitized T cells. Our results are not inconsistent with those demonstrating products of activated lymphocytes in supernates of T-cell-depleted lymphoid populations, but rather would argue that it may be
**Table I**

**Anti-DNP Antibody Response by Strain 2 and Strain 13 Guinea Pigs Immunized with DNP-PLL-Ova in CFA**

| Strain | No. of animals | Binding of [3H]DNP-Lysine by antiserum dilution* |
|--------|----------------|-----------------------------------------------|
| 2      | 5              | 55 ± 5, 15 ± 2                                 |
| 13     | 5              | 56 ± 4, 9 ± 2                                  |

* Serum anti-DNP antibody is expressed as percent binding of 0.1 ml 10⁻⁶ M [3H]DNP-lysine by 0.1 ml of the diluted serum. The arithmetic mean and standard error are shown.

**Table II**

**MIF Production by Strain 2 and Strain 13 Guinea Pig Spleen Cells Stimulated with DNP-PLL and DNP-PLL-Ova**

**Table III**

**T-Cell Responses of Lymph Node Cells of Strain 2 and Strain 13 Guinea Pigs Immunized with DNP-PLL-Ova Measured by the Virus Plaque Assay**

- **V-PFC/10⁶ cells**
- **ΔV-PFC/10⁶ cells**

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* Calculation in five of six experiments was made by comparing migration of supernates of sensitized cells cultured with antigen with reconstituted supernates of cells being cultured without antigen, antigen being reconstituted after removal of cells. In one experiment the controls consisted of nonsensitized spleen cells cultured with antigen. Since there was no significant difference in the two methods, the data were pooled.

* A single representative experiment is presented; four experiments using this assay yielded similar results.
difficult, if not impossible, to remove all competent T cells by physical separation methods. It may be recalled that as few as 0.6% immune lymphocytes produced migration inhibition of normal peritoneal cells (22); similarly, small numbers of contaminating T cells may be sufficient to activate B cells to produce MIF. The data would suggest that B cells may require two signals for activation to produce MIF, binding of antigen to their receptor and a second signal produced by T cells in this case.

The most difficult data to reconcile with these conclusions are those of Rocklin et al. (3) who reported that human MIF was produced by purified B as well as T cells. There are several possible explanations for the discrepancies: (a) their method for purifying B lymphocytes, i.e. adherence to anti-Ig columns, may retain some T cells, possibly activated T-cells containing Fc-receptors which could have been scored as B cells after elution with Ig; or (b) binding of B cells to the anti-Ig columns may lower the threshold for activation of B cells by antigen to produce MIF, analogously to the demonstration by Wahl et al. (5) that immune complexes can activate nonimmune B lymphocytes to produce macrophage chemotactic factor.

In the present experiments, MIF production by immune spleen cells was carrier specific in that DNP-GPA failed to elicit MIF in either strain 2 or 13 animals. Although the carrier specificity of the direct migration inhibition test was demonstrated in 1964 by David et al. (16), those experiments were carried out on immune peritoneal exudate cells, a population deficient in B cells which might have been capable of responding to hapten on heterologous carriers. A similar concern applies to the interpretation of direct migration inhibition studies using DNP-PLL (17).

There are a number of reservations about extrapolating from our results to those in other systems. First, it is not inconceivable that differences in patterns of response may exist between guinea pig and human lymphocyte populations. Secondly, the existence of a specific T-cell defect in strain 13 guinea pigs, with respect to DNP-PLL, does not exclude the possibility of additional Ir gene-controlled defects at the B-cell level as well. Although it has been demonstrated that the number of specific antigen-binding B lymphocytes does not differ between responder and nonresponder guinea pigs (18) and that the anti-DNP antibody clones appear to be identical (19), recent studies of Ir-gene controlled responses in certain mouse strains have raised the possibility that some nonresponder strains may have defects in both B- and T-cell populations (20). An Ir-gene controlled restriction on the ability of strain 13 B cells to respond to DNP-PLL cannot be ruled out at this time.

**Summary**

The question whether B lymphocytes are capable of being activated by antigen in the absence of functional T cells was investigated in a model that excludes participation of T cells by virtue of an immune response gene restriction. Strain 2 guinea pigs are capable of responding to immunization with DNP-PLL, whereas strain 13 animals are not. In the present experiments, animals of both strains were immunized with DNP-PLL complexed to ovalbumin (DNP-PLL-Ova) under conditions in which equal titers of antibodies to DNP were
produced by both strains. The failure of T cells of strain 13 animals to respond to DNP-PLL was confirmed by the virus plaque assay. While spleen cells from both strains produced MIF after stimulation with DNP-PLL-Ova, in response to DNP-PLL only strain 2 spleens were able to produce MIF. Cells from neither strain could be activated by DNP-guinea pig albumin to produce MIF.

We conclude that B lymphocytes are incapable of being stimulated by antigen in the absence of T cells, and that MIF production is a thymus-dependent response. While the results indicate that MIF production is a valid qualitative assay for T-cell competence, since MIF can be produced by B and T cells, the degree of migration inhibition cannot be regarded as a quantitative measure of T-cell function.

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