Delayed expulsion of the nematode *Nippostrongylus brasiliensis* from rats on a low protein diet: the role of a bone marrow derived component1–3

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ABSTRACT Rats on a low protein diet, containing 10% casein as the only source of protein, have an impaired capacity to expel primary infections with the nematode *Nippostrongylus brasiliensis* and remain susceptible to reinfection. In the present study, the transfer of syngeneic bone marrow cells to rats on a low protein diet reconstituted the expulsion mechanism allowing parasite rejection to occur at the same rate as rats on a sufficient diet. Serum transfer, on the other hand, did not significantly alter the rate of worm expulsion. These results demonstrate that a bone marrow derived component plays an important role in the impaired immunity of rats fed a low protein diet. Am. J. Clin. Nutr. 34: 400-403, 1981.

KEY WORDS Protein deficiency, nippostrongylus, bone marrow

Synergism between infection and malnutrition is a major cause of morbidity and mortality in developing countries, and there is now abundant evidence that the immune response is impaired in malnourished individuals (1, 2). Although immunological abnormalities are widespread in protein-calorie malnutrition and involve multiple components of the immune response, the functional significance of these various defects is not clear.

In previous studies of rats fed a low protein and/or iron-deficient diet and then infected with the nematode *Nippostrongylus brasiliensis*, natural expulsion of the parasite (3) and development of acquired resistance to reinfection (4) were significantly impaired. Since both of these functions have a recognized immunological basis (5, 6) it is reasonable to assume that their impairment in nutritionally deficient animals is a consequence of an impaired immune response.

In an earlier study designed to determine the role of cell-mediated immunity in rats on a low protein and iron-deficient diet, syngeneic transfer of immune lymphocytes did not reconstitute the expulsion mechanism in deficient animals (7). This suggested that the production of sensitized lymphocytes occurs normally in low protein and iron-deficient animals and that some other component of the immune response is defective. The aim of this study was to determine whether the transfer of serum or syngeneic bone marrow cells would affect the rate of worm expulsion in rats on a low protein diet. The term syngeneic implies genetic homozygosity, and thus cells transferred from one animal to another are identical genetically. This requirement is essential, otherwise homograft rejection would interfere with the function of transferred cells. The life cycle of this parasite and aspects of the immune response have been summarized in earlier studies (3, 4).

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Materials and methods

Animals
Donor and recipient animals were highly inbred DA rats maintained by line mating (8). The guidelines for the care and use of animals for research purposes as set out by the National Health and Medical Research Council were followed.

Parasite
Methods of culture and administration of infective larvae have been described previously (9).

Preparation of diets
Purified diets were prepared which were free of amino acids and contained either 10 or 30% casein as the sole protein source. The casein was prepared by acid hydrolysis and produced by Mead Johnson (Australia).

Immunization of cell donors
Donors prepared on a sufficient diet (30% casein) were infected twice with 1000 larvae of *N. brasiliensis* at 3 and 1 wk before preparation of cell suspensions.

Preparation and transfer of bone marrow cells
The method described by Dineen and Kelly (8) was followed. Femurs and tibiae were dissected out and stripped free of adherent muscle and connective tissue. The ends were cut off and the bone marrow washed out with Hanks' solution, using a 10-ml syringe fitted with a 23-gauge needle. Bone marrow cells were dispersed by dicing with fine scissors and then drawing up and down using a wide mouthed pipette. The cells were then filtered through sterile gauze, washed in chilled Hanks' solution by centrifugation, and then resuspended in Hanks' solution and counted. Cells were injected into the lateral tail vein of recipients within 2 h of collection.

Preparation and transfer of serum
Blood was collected from anaesthetised rats by aortic puncture. Serum was then pooled and administered by injection into the lateral tail vein of recipients.

Statistics
Body weight, haemoglobin and albumin were analyzed by Student's *t* test. Analysis of variance was carried out on all worm count data after transformation to log_{10}(X + 1).

Experimental design and results

Transfer of serum and syngeneic bone marrow cells
Twenty DA rats (groups 1 to 4) were weaned onto low protein diets for use as cell or serum recipients. Two further groups, one on a sufficient diet (group 5) and one on a low protein diet (group 6) acted as infection controls. There were two donor groups, each of 10 animals, and rats in one of these groups were infected twice with *N. brasiliensis* (see "Materials and methods") to provide immune serum and bone marrow cells.

At 10 wk of age all animals were weighed and blood was collected for hemoglobin estimation. These results, together with the serum albumin levels which were measured on blood collected at the time of autopsy, are presented in Table 1. There was no significant difference between the serum albumin levels performed before the experiment in the case of the donor animals and after the experiment in the case of recipient rats and so the combined results are presented. Rats on the 10% protein diet had significantly lower body weight and serum albumin levels than rats on the 30% protein diet.

On day 0 group 1 rats were given 120 × 10^6 immune bone marrow cells by intravenous injection, and group 2 rats received 120 × 10^6 nonimmune bone marrow cells intravenously. Groups 3 and 4 were given 1 ml of immune and nonimmune serum, respectively, by the intravenous route. On the same day rats in all groups were inoculated with 1000 larvae of *N. brasiliensis*.

All animals were autopsied for total worm counts on day 16, and these results are presented in Table 2. There was a marked delay in parasite expulsion from nonrecipient rats on a low protein diet (group 6, mean worm count 323) when compared with those on a sufficient diet (group 5, mean worm count 0; group 5 versus group 6, *p* < 0.01), thus confirming delayed expulsion from rats on a low protein diet.

The transfer of serum, whether immune or nonimmune, did not significantly alter the rate of parasite expulsion in rats on a low protein diet (groups 3 and 4, mean worm count 262 and 281, respectively; groups 3 and 4 versus group 6, *p* > 0.05). In contrast, syngeneic bone marrow cell transfer, whether

| TABLE 1 |
| --- | --- | --- |
| Dietary status & 10% Protein & 30% Protein & *p* |
| Body weight (g) & 83.9 ± 4 & 163.6 ± 7 & <0.01 |
| Albumin (g/l) & 25.0 ± 2 & 30.9 ± 1 & <0.01 |
| Hemoglobin (g/dl) & 12.9 ± 1 & 13.7 ± 0 & NS |
TABLE 2
The effect of serum and syngeneic bone marrow cell transfer on expulsion of *N. brasiliensis* from rats on a low protein diet

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Transferred component</th>
<th>Total worm count (mean ± SE)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Experimental</td>
<td>10.2 ± 4</td>
<td>Groups 1 and 2</td>
</tr>
<tr>
<td>2</td>
<td>Immune bone marrow cells</td>
<td>1.7 ± 1</td>
<td>vs. group 5 NS</td>
</tr>
<tr>
<td>3</td>
<td>Nonimmune bone marrow cells</td>
<td>262.3 ± 51</td>
<td>Groups 3 and 4</td>
</tr>
<tr>
<td>4</td>
<td>Immune serum</td>
<td>281.4 ± 57</td>
<td>vs. group 6 NS</td>
</tr>
<tr>
<td>5*</td>
<td>Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Nil</td>
<td>323.0 ± 31</td>
<td></td>
</tr>
</tbody>
</table>

* Group 5 was on a protein-sufficient diet; all other groups were on a low protein diet.

Constituted the expulsion mechanism in low protein diet rats. The nature of the bone marrow derived component was not defined in this study, but may well involve immunologically nonspecific components, as both immune and nonimmune bone marrow cells were equally effective.

In addition to studies demonstrating the role of bone marrow cells in expulsion of *N. brasiliensis* from the rat (6, 8), bone marrow cells are also involved in expulsion of *Trichinella spiralis* (12) and *Listeria monocytogenes* (15) from the mouse. The bone marrow cell line involved has not been unequivocally defined in any system, although the monocyte has been implicated in the case of *L. monocytogenes* (14, 15). As well as noncellular components such as iron and folate, bone marrow transfer would provide white cell precursors of lymphocyte, granulocyte, and monocyte populations. It is probable that the component from bone marrow which reconstitutes worm expulsion is nonlymphoid in nature, since lymphocytes from peripheral lymph nodes were unable to restore the immune response in iron and protein-deficient rats (7).

It may be that the essential component provided by bone marrow is the granulocyte, since it has been suggested that biogenic amines play a role in the expulsion of *N. brasiliensis* (16, 17). The evidence implicating a myeloid-amine response, however, is largely circumstantial. For example, although the histamine antagonist promethazine hydrochloride delays expulsion of *N. brasiliensis* (16), later studies demonstrated that promethazine has immunosuppressant as well as anti-histaminic properties (11, 18). Evidence against a role for impaired granulocytes mediating delayed parasite expulsion has been provided by Wells (19). She demonstrated that mast cells in the small intestine of rats infected with *N. brasiliensis* were equal in number in both protein sufficient and low protein diet rats, and although eosinophils were less common in low protein diet rats, the intestinal histamine content was higher.

The other white cell precursor present in the bone marrow is the monocyte. Cooperation between the monocyte and immunologically committed lymphocytes is important in delayed type hypersensitivity (20). Thus thymectomized irradiated rats require mon-
ocytes as well as lymphocytes to express tuberculin sensitivity (21).

The present results give a strong indication that a bone marrow derived component is defective in rats on a low protein diet. Reconstitution studies in lethally irradiated recipient rats with lymphocytes and bone marrow from protein deficient and sufficient rats are currently underway to further define the nature of the low protein-induced defect in worm expulsion.

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References