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Role of Thymus for *N*-Acetyl Muramyl-L-Alanyl-D-Isoglutamine-Induced Polyarthritis and Granuloma Formation in Euthymic and Athymic Nude Rats or in Neonatally Thymectomized Rats

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A synthetic adjuvant, *N*-acetyl muramyl-L-alanyl-D-isoglutamine (MDP), produced extremel severe polyarthritis with almost 100% incidence in Rowett euthymic rnu/+ rats, but the same dose of MDP (100 μg) did not produce the disease in athymic rnu/rnu rats. Five hundred micrograms of MDP or 0.2 mg of heat-killed *Mycobacterium bovis* BCG, however, produced mild and transient polyarthritis in nude rats with very low incidence. We have not yet succeeded in reconstituting the disease susceptibility of nude rats by using thymus cells from normal rnu/+ rats. After intradermal inoculation of 100 μg of MDP, nude rats developed small granulomas with a little necrosis and very few multinucleated giant cells only in the regional lymph nodes, whereas, in addition to the development of polyarthritis, euthymic rnu/+ rats developed typical granuloma with massive necrosis accompanied by numerous polymorphonuclear leukocytes and sparse multinucleated giant cells in the regional lymph nodes. Thymus cell-reconstituted rnu/rnu rats developed granuloma with sparse giant cells, relatively large areas of necrosis, and many polymorphonuclear leukocytes. Neonatal thymectomy may depress adjuvant-induced arthritis in the high-responder Lewis rats and enhance the disease development in the low-responder F344 rats. These findings suggested that (i) thymus plays an important role in promoting the development of MDP-induced arthritis; (ii) MDP-induced granuloma formation does not require thymus functions; (iii) the thymus functions may however be involved in the development of massive necrosis surrounded by considerable polymorphonuclear leukocyte infiltration, the mechanisms of which remain to be determined; and (iv) there is no direct correlation between granuloma formation and development of adjuvant arthritis.

Adjuvant-induced arthritis can be produced by a single injection of Freund complete adjuvant containing *Mycobacterium* (20). Various bacteria, their cell walls, and their peptidoglycans (12) can be substituted for *Mycobacterium* in Freund complete adjuvant. Our recent findings (14) revealed that a synthetic adjuvant, *N*-acetyl muramyl-L-alanyl-D-isoglutamine (MDP), was able to produce polyarthritis in high-responder Lewis rats comparable to that produced by Freund complete adjuvant. MDP-induced arthritis was indistinguishable from adjuvant-induced arthritis in terms of clinical course and chronic granulomatous lesions around joints, including periarticular tissue and skin nodules (14, 21).

Adjuvant-induced arthritis has been thought to be caused by a cell-mediated immune response, one manifestation of delayed-type hypersensitivity to mycobacterial fragments (23, 30), or by some host tissue component, such as collagen (26). The role of cell-mediated immune response mechanisms responsible for development of arthritis has been supported by the successful adoptive transfer of this disease with thoracic duct cells (31), spleen cells, and lymph node cells (22). Recently, a thymus-derived suppressor cell population has also been postulated (10), further suggesting the importance of cell-mediated immune mechanisms. There have been, however, conflicting results with regard to the effect of neonatal thymectomy on production of adjuvant-induced arthritis. Neonatal thymectomy markedly inhibited the development

of adjuvant-induced arthritis (17), whereas Lennon and Byrd (16) and others (1) claimed no difference in disease susceptibility between neonatal thymectomized and sham-thymectomized rats. In this regard, athymic nude rats would provide us a very nice opportunity to investigate whether or not the thymus functions could be involved in development of this disease.

Emori and Tanaka (7) found that MDP produced epithelioid granuloma in the regional lymph nodes indistinguishable from that produced by *Mycobacterium* and suggested that MDP is one of the granulomagenic components of *Mycobacterium*. They also suggested a very close correlation between adjuvancy and granulomagenicity of MDP. These findings prompted us to consider the possibility that granuloma formation may play a key role in an immunopathological process of this disease development. It is also important to elucidate how thymus functions can regulate the granuloma formation in addition to modulating the development of this disease in rnu/rnu and rnu/+ rats. Furthermore, there are several advantages in investigating an immunopathological event of MDP-induced arthritis because MDP is a totally synthesized molecule (18), has a low molecular weight and is nonantigenic (3), and displays little or no mitogenicity (19).

The aim of this present investigation was to determine the involvement of the thymus in development of this disease, using either neonatal thymectomized rats or Rowett nude rats, and also to elucidate a cause-effect relationship between granuloma formation and production of polyarthritis by MDP.

MATERIALS AND METHODS

Animals. Inbred Lewis and F344 rats were obtained from Microbiological Associates, Bethesda, Md. Rats were bred in the animal facilities of the Rehabilitation Center, University of California–Los Angeles. Both male and female offspring were used for neonatal thymectomy.

Rowett euthymic rats (rnu/+) and athymic nude rats (rnu/rnu) were obtained from the Experimental Animal Center, Kawasaki, Japan, where these strains have been bred under specific-pathogen-free conditions. Congenitally athymic nude (rnu/rnu) rats and their thymus-bearing littermates (rnu/+) were obtained by mating homozygous (rnu/rnu) males with heterozygous (rnu/+) females. All experimental procedures were carried out either in vinyl flexible isolators under specific-pathogen-free or clean bench conditions.

Neonatal thymectomy. Neonatal thymectomy was carried out according to the method of Jankovic et al. (9). Thymectomy was carried out at less than 12 h and at 36 h, 72 h, 4 days, 7 days, and 11 days after birth. All the thymectomized animals were immunized at 8 weeks old in both inguinal lymph nodes with 0.01 ml of a water-in-oil emulsion containing 0.1 mg of heat-killed mycobacteria (described below). Groups of rats were also sham-thymectomized by subjecting them to the same surgical procedure without removal of the thymus. At the end of the experiments, all the rats were sacrificed and examined macroscopically for the existence of thymus residues.

Adjuvants. A mixture of heat-killed *Mycobacterium tuberculosis* strains C, DT, and PN and heat-killed *Mycobacterium bovis* BCG was obtained through the courtesy of Fisheries Veterinary Laboratory, Weybridge, Surrey, England. A 4-mg sample of these bacteria was crushed well with a mortar and pestle in a few drops of the total 0.1 ml of heavy mineral oil (Squibb and Sons, Princeton, N.J.) containing 5% Arlacel A (Hilltop Laboratories, Cincinnati, Ohio) and emulsified by a dropwise addition of 0.1 ml of phosphate-buffered saline (0.02 M, pH 7.2). Wax D (peptidoglycolipid) was isolated and purified from human mixed strains of mycobacteria (C, DT, and PN) or from $H_{37}Rv$ (virulent strain of *M. tuberculosis*) according to the method of Asselineau et al. (2).

A synthetic adjuvant, MDP, was obtained from A. Inoue, Daiichi Pharmaceutical Co., Tokyo. A 4-mg sample of MDP dissolved in 1 ml of phosphate-buffered saline was sterilized by filtration through a membrane filter (Millipore Corp., Bedford, Mass.), pore size 0.22 μm, and emulsified with sterile Difco incomplete adjuvant (Difco Laboratories, Detroit, Mich.), which consists of 85% Bayol F and of Arlacel A, instead of the heavy mineral oil described above, according to our previous report (14). Rowett rnu/rnu and rnu/+ rats, under ether anesthesia in an isolator, were inoculated intradermally in the left hind footpad with 0.05 ml of a water-in-oil emulsion containing 0.1 mg of MDP.

Transfer of thymus cells. A normal female rnu/+ rat, 5 weeks old, was killed by cardiac puncture under ether anesthesia. The thymus was aseptically removed into cold Hanks balanced salt solution under clean bench conditions. Thymus cells were expressed through sterile stainless-steel mesh and diluted to make a suspension of 10^8 cells per ml. The thymus cells were tested for viability by exclusion of 0.2% trypan blue. Athymic female rats (5 weeks old) were injected intravenously with 0.2 ml of this thymus cell suspension. One day after thymus cell transfer, all the rats, athymic female rats and euthymic female rats, were injected intradermally in the left hind footpad with 0.05 ml of a water-in-oil emulsion containing 0.1 mg of MDP.

Adjuvant-induced arthritis. After inoculation, the rats were examined daily for at least 2 months to evaluate time of onset of polyarthritis and graded from 0 to 4 for each appendage, according to a previous paper (12).

Histological studies. Rats were sacrified at various intervals (7, 14, 21, and 28 days, etc.) after footpad injection of MDP. Tissue specimens taken were popliteal and inguinal lymph nodes, spleen, liver, left lung, kidney, thymus, and infected and contralateral footpad, etc. These specimens were fixed in 10% Formalin and stained with hematoxylin and eosin.

RESULTS

Effect of neonatal thymectomy on development of adjuvant-induced arthritis in low- and high-responder rats. As shown in Table 1, neonatal F344 rats thymectomized at 36 h, 4 days, and 11 days developed arthritis that had a higher incidence (33, 27, and 33%, respectively) but was not significantly more severe than that developed by sham-operated or normal F344 rats. Onset of the disease was slightly delayed in thymectomized rats. On the other hand, neonatal thymectomized Lewis rats developed the disease with slightly delayed onset day and rather less severity than that in normal Lewis rats.

Susceptibility of Rowett rnu/+ and rnu/rnu rats to MDP-induced polyarthritis. Our previous reports (11, 13) failed to demonstrate arthritogenicity of MDP even with high doses in high-responder rats. However, we found recently that MDP in Difco incomplete Freund adjuvant produced moderate to severe polyarthritis in Lewis rats (14), germfree F344 rats, and WKA rats (unpublished data). To assess the susceptibility of euthymic rnu/+ rats and congenitally athymic nude rnu/rnu rats to adjuvant-induced arthritis, MDP was mixed with Difco incomplete adjuvant to make a water-in-oil emulsion and injected intradermally in the left hind footpad. As shown in Table 2, rnu/+ rats developed moderate to severe arthritis with almost 100% incidence. The clinical signs appeared at 9 to 13 days after administration of 0.1 mg of MDP and gradually became severe, reached a maximal severity of 2 to 16 at 14 to 17 days, and were still observed as moderate to severe lesions until the rats were sacrificed at 60 days after injection. Heat-killed BCG induced extremely severe arthritis. Freund incomplete adjuvant did not induce any disease during the 2 months of observation. In contrast, athymic nude rats failed to develop polyarthritis during the 3 months of observation after administration of 0.1 or 0.2 mg of MDP. After inoculation of 0.2 mg of heat-killed BCG or 0.5 mg of MDP, the nude rats developed very mild and transient arthritis. In these cases, the clinical signs appeared at 19 to 24 days only in the hind footpad and subsided 5 to 7 days later. To assess thymus reconstitution, nine athymic rnu/rnu female rats (5 weeks old) received 1.6×10^7 normal thymus cells intravenously from one normal female rnu/+ rat (5 weeks old). One day later, these thymus cell-transferred rnu/rnu rats were injected intradermally with 0.05 ml of MDP into the left hind footpad. At the same time, euthymic rnu/+ rats (5 weeks old) were also injected intradermally with 0.1 mg of MDP. All eight rnu/+ rats developed severe arthritis, whereas none of the thymus cell-transferred rnu/rnu rats developed the disease. In another experiment, 10 rnu/rnu rats received two consecutive injections of thymus cells from normal rnu/+ rats and were dosed with MDP as described above. None of them developed the disease.

MDP-induced granuloma of the regional lymph nodes in rnu/rnu and rnu/+ rats. After injection of a water-in-oil emulsion of 0.1 mg of MDP, both rnu/rnu and rnu/+ rats developed typical granuloma in the regional lymph nodes, such as the popliteal and inguinal lymph

TABLE 1. *Effect of neonatal thymectomy on adjuvant-induced arthritis in high-responder Lewis rats and low-responder F344 rats*

Time of neonatal thymectomy[a]	Injected material[b]	Incidence of arthritis/total (%)	Mean onset day (onset day)	Mean severity[c]
F344 rats				
Within 36 h	$H_{37}Rv$ Wax D	3/10 (33)	13.3 (11–17)	13.3
Day 4		3/11 (27)	13.3 (11–15)	8.7
Day 11		4/12 (33)	15.3 (12–18)	8.2
Sham-operated		1/10 (10)	11.0	9.0
Normal control		3/20 (15)	10.5 (10–13)	8.6
Lewis rats				
Within 12 h	C, DT, and PN	19/19 (100)	10.0 (7–17)	12.6
Day 3		16/16 (100)	9.4 (7–13)	15.5
Day 7		9/9 (100)	9.7 (7–11)	17.1
Normal control		8/8 (100)	8.0 (7–12)	17.4

[a] Neonatal thymectomy was carried out at the various times after birth as indicated.

[b] Each rat, thymectomized, sham-thymectomized, or normal, was injected in both inguinal lymph nodes with 0.5 mg of $H_{37}Rv$ Wax D or heat-killed *M. tuberculosis* (mixed strains of C, DT, and PN) at 8 weeks old.

[c] Mean severity was calculated as the arithmetic mean of the highest score of each rat per group, only from positive rats.

TABLE 2. *Susceptibility of Rowett rnu/+ and rnu/rnu rats to MDP-induced or heat-killed BCG-induced polyarthritis*

Rats	Injection at age[a] (wk)	Adjuvant	Dose (μg per rat)	Polyarthritis			
				Incidence/ total	Onset day (mean)	Severity[b] (mean)	
Rowett rnu/+	5	MDP	100	8/8	9–13 (10.0)	2–16	(10.2)
	8	MDP	100	7/8	9–11 (10.2)	13–16	(14.5)
	5	BCG[c]	200	9/9	9–10 (10.9)	16.0	
	5	FIA[d]		0/9			
Rowett rnu/rnu	5	MDP	100	0/6			
	5	MDP	100	0/9			
	6	MDP	100	0/8			
	8	MDP	200	0/4			
	5	MDP	500	2/9	19–22 (20.0)	2–8	(5.0)
	8	BCG	200	2/13	23–24 (23.5)	2–5	(3.5)
	5	FIA		0/7			
Lewis	5	MDP	100	6/10	10–14 (12.5)	3–11	(7.3)
	8	MDP	100	13/20	10–18 (16.0)	1–16	(7.0)

[a] Each rat was injected intradermally into the left hind footpad with 0.05 ml of a water-in-oil emulsion containing the indicated amounts of various adjuvants at the indicated age.
[b] See Table 1, footnote *c*.
[c] Heat-killed BCG (see text).
[d] Freund incomplete adjuvant (Difco incomplete adjuvant).

nodes (Fig. 1 and 2), whereas granuloma formation was not observed in the contralateral lymph nodes (data not shown). Athymic nude rats developed granuloma in small size with a little necrosis surrounded by a few polymorphonuclear leukocytes (PMNs) (Fig. 3). In contrast, euthymic rnu/+ rats developed very large granulomas with massive necrosis in the center of the granuloma, considerable PMN infiltration, and sparse multinucleated giant cells (Fig. 4). Granuloma formations were also observed in the liver and spleen of rnu/+ rats, but not in rnu/rnu rats (data not shown). A water-in-oil emulsion without MDP, as an oil control, induced small and mild reticulosis only around oil droplets deposited in the regional lymph nodes of rnu/rnu rats (Fig. 5). It should be noted that the thymus cell-transferred rnu/rnu rats described above developed granuloma with relatively large areas of necrosis surrounded by relatively heavy PMN infiltration and few giant cells (Fig. 6). One rnu/rnu rat developed a nearly typical granuloma at 42 days after administration of MDP, with a large area of necrosis surrounded by many PMNs and giant cells, as observed in Fig. 4.

DISCUSSION

The present studies demonstrated that neonatal thymectomy may depress adjuvant-induced arthritis in the high-responder Lewis rats and enhance the disease development in the low-responder F344 rats. These findings did not prove, but suggested, the regulatory role of the thymus in promoting the development of the disease. The most important finding of the present studies was that euthymic rnu/+ rats developed very severe polyarthritis after injection of 0.1 mg of MDP, whereas athymic nude rnu/rnu rats did not develop the disease with up to 0.2 mg of MDP. This finding suggests the very important role of thymus for promoting the development of adjuvant-induced arthritis. When the dose of MDP was increased, the nude rats developed very mild and transient arthritis with low incidence and only in the restricted hind footpad.

If adjuvant-induced arthritis is a T-cell- or thymus-associated disease as postulated earlier (10, 22, 23), one can consider that the development of arthritis in nude rats even with very low incidence and transient lesions suggests the existence of T-cell lineage in Rowett rnu/rnu rats. Rowett nude rats are known to be in many ways similar to nude mice, including reported failure to reject skin allografts, the absence of a delayed-type hypersensitivity reaction to tuberculin, the inability to generate an immunoglobulin M and G response to thymus-dependent antigens, and unresponsiveness of spleen cells upon stimulation with mitogens (29). However, it is also accepted that nude mouse spleen cells contain pre-T cells (25), which can be differentiated into functioning mature T cells (4, 24). If this is the case in nude rats, an alternative possibility is that Rowett nude rats may possess precursors of T cells, or pre-T cells, and that these pre-T cells

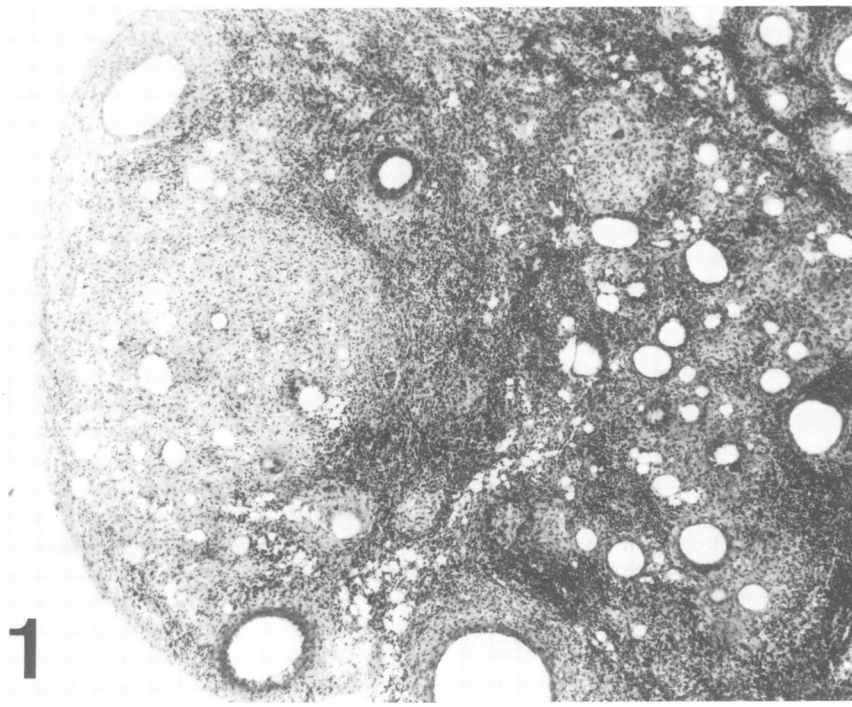

FIG. 1. *Left popliteal lymph node in rnu/rnu rat, 16 days after administration of MDP. Granulation tissue around oil droplets, with little or no necrosis infiltrated by very few PMNs and very few multinucleated giant cells (see Fig. 3). Hematoxylin and eosin; ×60.*

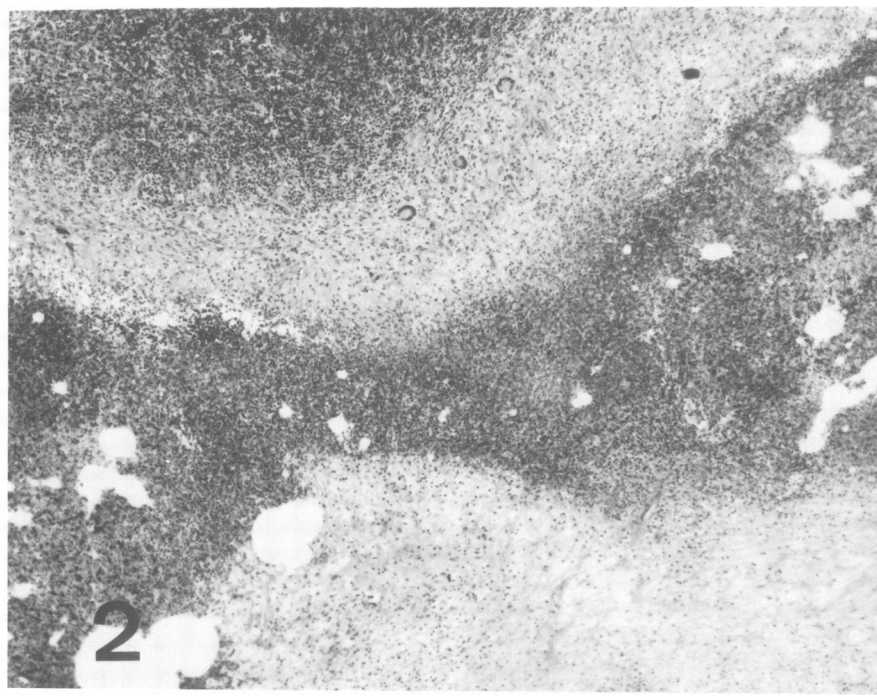

FIG. 2. *Left popliteal lymph node in rnu/+ rat, 16 days after administration of MDP. Massive necrosis and heavy PMN infiltration, surrounded by granulation tissue growth, characteristic of epithelioid cells, and sparse giant cells (see Fig. 4). Hematoxylin and eosin; ×60.*

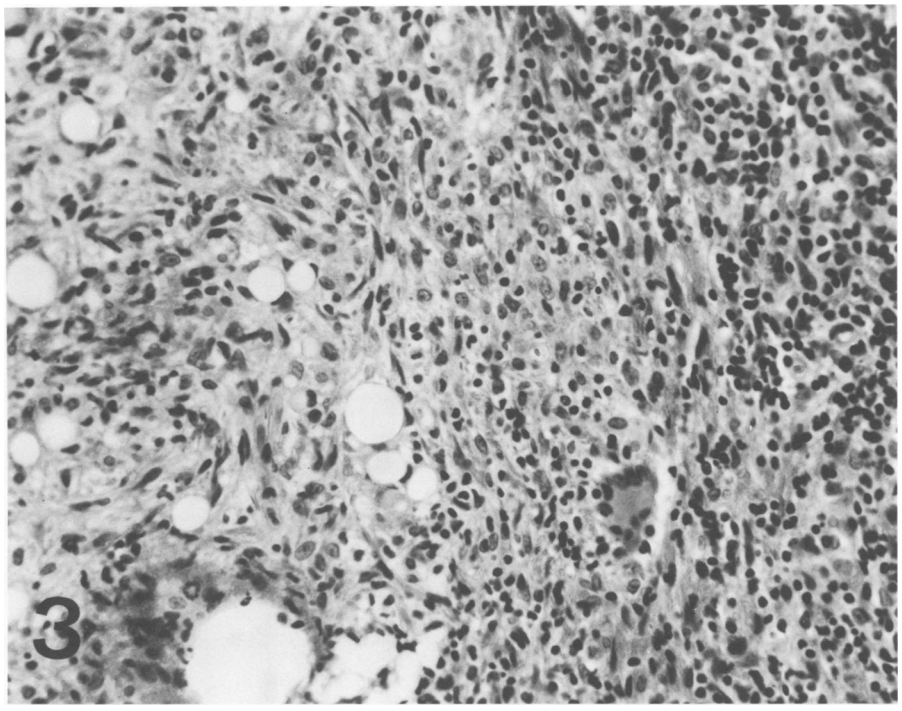

FIG. 3. *Poor granulation tissue in small size around oil droplets with very few tiny areas of necrosis, infiltrated by very few PMNs and very few giant cells. Enlargement of Fig. 1. Hematoxylin and eosin; ×300.*

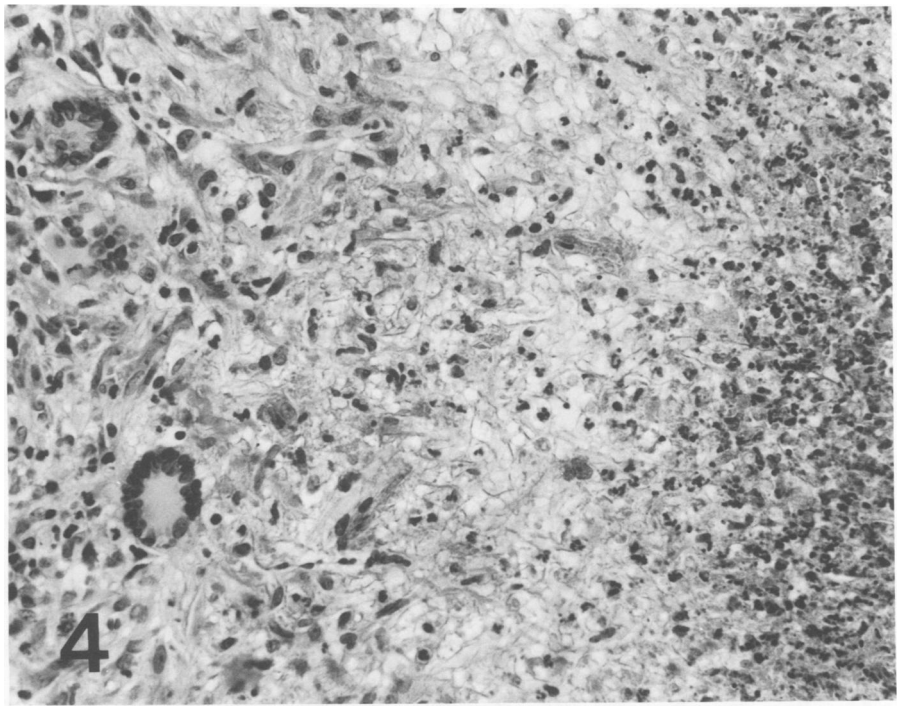

FIG. 4. *Typical granuloma formation with sparse multinucleated giant cells, epithelioid cells, and fibroblast proliferation. Note numerous PMNs, mostly neutrophils, in the massive necrosis at the right edge of the photograph. Enlargement of Fig. 2. Hematoxylin and eosin; ×300.*

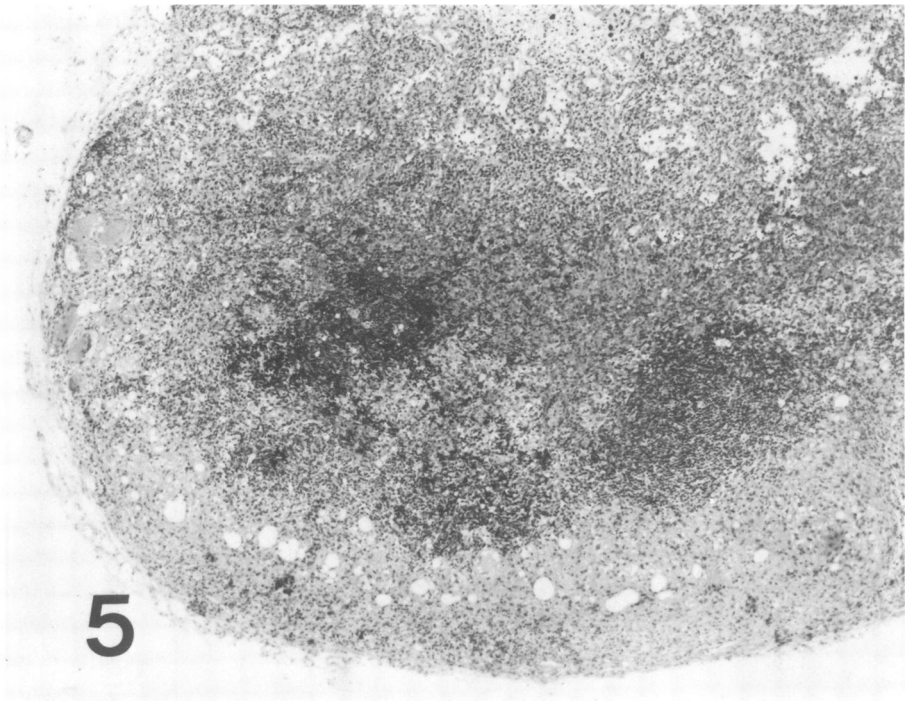

FIG. 5. *Left popliteal lymph node in rnu/rnu rat, 20 days after administration of water-in-oil emulsion without MDP. Very mild granulation tissue reaction, only around oil droplets. Hematoxylin and eosin; ×60.*

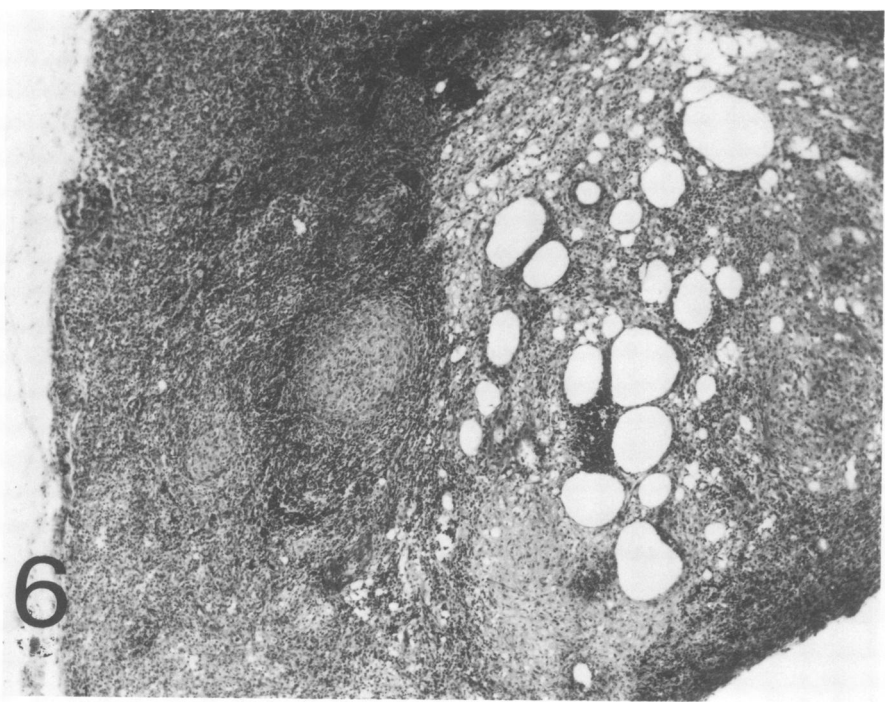

FIG. 6. *Left popliteal lymph node in thymus-reconstituted rnu/rnu rat, 20 days after administration of MDP. Note relatively large area of necrosis, heavy PMN infiltration, and sparse giant cells. Hematoxylin and eosin; ×60.*

can be stimulated by strong adjuvants such as BCG or MDP to become functioning mature T cells, as postulated for nude mice (4, 24). This possibility remains to be solved in future studies.

Rowett rnu/+ rats were proved to be one of the most susceptible strains for MDP-induced arthritis among Lewis (60 to 80% incidence), Wistar (50 to 70%), Sprague-Dawley, F344, and Rowett rats (our unpublished data). We have been interested in knowing whether or not the thymus from rnu/+ rats can reconstitute the susceptibility of nude rats for development of MDP-induced arthritis. In our limited experiments, thymus cell suspensions of female rnu/+ rats failed to reconstitute high susceptibility to the disease in nude rats. This failure of thymus reconstitution does not necessarily mean thymus independence of development of MDP-induced arthritis. Further studies are required with regard to dose responses to the transferred cells, time schedules between thymus cell transfer and administration of MDP, and thymus graft experiments, etc. Our preliminary data indicate that all of the thymus cell-transferred nude rats developed very severe arthritis similar to that of rnu/+ rats when they were injected with 0.2 mg of heat-killed BCG 4 weeks after thymus cell transfer.

On the other hand, MDP could induce epithelioid cell granuloma in both euthymic and athymic rats even though somewhat different histological features existed in each type of rat. The rnu/+ rats developed a typical epithelioid granuloma consisting of well-developed epithelioid cell and multinucleated giant cells, not only in the regional lymph nodes (Fig. 2) but also in liver and mesenteric omentum adhered over the spleen (data not shown). As a prominent feature, this granuloma consisted of massive necrosis infiltrated with numerous PMNs in the center of the granuloma (Fig. 4), so that this granuloma can be considered a necrotizing epithelioid granuloma. In contrast, athymic rats developed epithelioid granuloma only in the regional lymph nodes with little, if any, necrosis, surrounded by very few PMNs and multinucleated giant cells. Thymus cell-transferred athymic nude rats developed granulomas consisting of massive necrosis infiltrated with many PMNs, similar to that of rnu/+ rats described above. It is thus likely that the thymus functions are involved, by still unidentified pathways, in the pathogenesis of massive necrosis infiltrated with numerous PMNs. The appearance of multinucleated giant cells seems, however, unlikely to be associated with thymus functions, in contrast to the previous report (8, 28), because the giant cells could be induced in nude rats as well as in rnu/+ rats (Fig. 3 and 4). The reason why the transferred thymus cells can evoke such a central necrosis with heavy PMN infiltration remains to be discovered. We cannot rule out the possibility that thymus-dependent immune mechanisms may be involved in the production of the necrotizing epithelioid granuloma, since Unanue and Benaceraff pointed out the pathophysiological role of cell-mediated immunity for development of granuloma reaction (28). Although MDP-induced granuloma formation was not observed in the contralateral lymph nodes in either euthymic or athymic rats, one can consider that MDP-induced granuloma formation is due not only to the direct action of MDP deposited in the lymph nodes but also to thymus-dependent immune mechanism(s) responding either to MDP by itself or to still-undetermined host tissue components, possibly through a variety of biological activities of MDP such as enhancement of humoral and cell-mediated immune response (5), activation of macrophages (27), pyrogenicity (15), activation of complement, increasing vascular permeability, and plasma-dependent chemotactic activity to rat peritoneal macrophages and PMNs (our unpublished data).

It is thus concluded that (i) thymus plays an important role in promoting the development of MDP-induced arthritis; (ii) MDP-induced granuloma formation does not require thymus functions; but (iii) thymus functions may be involved in the development of massive necrosis infiltrated with numerous PMNs. Further studies would be required to elucidate how the thymus is involved in the pathogenesis of the MDP-induced arthritis and granuloma formation. The use of nude rats and rnu/+ rats is a powerful tool to elucidate the mechanisms of this disease production.

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LITERATURE CITED

1. **Arnason, B. G., and B. D. Jankovic, B. H. Waksman, and C. Wennersten.** 1962. Role of the thymus in immune reactions in rats. II. Suppressive effect of thymectomy at birth on reactions of delayed (cellular) hypersensitivity and the circulating small lymphocyte. J. Exp. Med. **116**:177-189.
2. **Asselineau, J., N. Choucroun, and E. Lederer.** 1950. Sur la constitution chimique d'um lipo-polysaccharide antigenique extrait de Mycobacterium tuberculosis var. hominis. Biochim. Biophys. Acta **5**:197-203.
3. **Audibert, F., B. Heymer, C. Gros, K. H. Schleifer, P. H. Seidl, and L. Chedid.** 1978. Absence of binding of MDP, a synthetic innumoadjuvant, to antipeptidoglycan antibodies. J. Immunol. **121**:1219-1222.
4. **Blankwater, M.-J., L. A. Levert, A. C. W. Swart, and D. W. van Bekkun.** 1978. Effect of various thymic and non-thymic factors on in vitro antibody formation by

spleen cells from nude mice. Cell. Immunol. **35**:242–252.
5. **Chedid, L., F. Audibert, P. Lefrancier, J. Choay, and E. Ledere.** 1976. Modulation of the immune response by a synthetic adjuvant and analogs. Proc. Natl. Acad. Sci. U.S.A. **73**:2472–2475.
6. **Currey, H. L. F., and M. Ziff.** 1968. Suppression of adjuvant disease in the rat by heterologous antilymphocyte globulin. J. Exp. Med. **127**:185–203.
7. **Emori, K., and A. Tanaka.** 1978. Granuloma formation by synthetic bacterial cell wall fragment: muramyl dipeptide. Infect. Immun. **19**:613–720.
8. **Hsu, C.-K., and S. H. Hsu.** 1976. Immunopathology of schistosomiasis in athymic mice. Nature (London) **262**: 397–399.
9. **Jankovic, B. D., B. H. Waksman, and B. G. Arnason.** 1962. Role of the thymus in immune reactions in rats. I. The immunologic response to bovine serum albumin (antibody formation, arthus reactivity, and delayed hypersensitivity) in rats thymectomized and splenectomized at various times after birth. J. Exp. Med. **116**: 159–175.
10. **Kayashima, K., T. Koga, and K. Onoue.** 1976. Role of T lymphocytes in adjuvant arthritis. I. Evidence for the regulatory function of thymus-derived cells in the induction of the disease. J. Immunol. **117**:1878–1882.
11. **Kohashi, O., S. Kotani, T. Shiba, and A. Ozawa.** 1979. Synergistic effect of polyriboinosinic acid: poliribocytidylic acid and either bacterial peptidoglycans or synthetic *N*-acetylmuramyl peptides on production of adjuvant-induced arthritis in rats. Infect. Immun. **26**:690–697.
12. **Kohashi, O., C. M. Pearson, Y. Watanabe, and S. Kotani.** 1977. Preparation of arthritogenic hydrosoluble peptidoglycans from both arthritogenic and nonarthritogenic bacterial cell walls. Infect. Immun. **16**: 861–866.
13. **Kohashi, O., C. M. Pearson, Y. Watanabe, S. Kotani, and T. Koga.** 1976. Structural requirements for arthritogenicity of peptidoglycans from *Staphylococcus aureus* and *Lactobacillus plantarum* and analogous synthetic compounds. J. Immunol. **116**:1635–1639.
14. **Kohashi, O., A. Tanaka, S. Kotani, T. Shiba, S. Kusumoto, K. Yokogawa, S. Kawata, and A. Ozawa.** 1980. Arthritis-inducing ability of a synthetic adjuvant, N-acetylmuramyl peptides, and bacterial disaccharide peptides related to different oil vehicles and their composition. Infect. Immun. **29**:70–75.
15. **Kotani, S., Y. Watanabe, T. Shimono, K. Harada, T. Shiba, S. Kusumoto, K. Yokogawa, and M. Taniguchi.** 1976. Correlation between the immunoadjuvant activities and pyrogenicities of synthetic N-acetylmuramyl peptides and amino acid. Biken J. **19**:9–13.
16. **Lennon, V. A., and W. J. Byrd.** 1973. Experimental arthritis in thymectomized rats with an impaired humoral immune response. Nature (London) **244**:38–40.
17. **Lujan, M. A., and B. Candiolo.** 1965. Influence of thymectomy on experimental polyarthritis. Acta Physiol. **15**:33–37.
18. **Merser, C., P. Sinay, and A. Adam.** 1975. Total synthesis and adjuvant activity of bacterial peptidoglycan derivatives. Biochem. Biophys. Res. Commun. **66**:1316–1322.
19. **Parant, M., C. Damais, F. Audibert, F. Parant, L. Chedid, E. Sache, P. Lefrancier, J. Choay, and E. Lederer.** 1978. In vivo and in vitro stimulation of nonspecific immunity by the β-D-*p*-aminophenylglycoside of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) and an oligomer prepared by cross-linking with glutaraldehyde. J. Infect. Dis. **138**:378–386.
20. **Pearson, C. M.** 1956. Development of arthritis, periarthritis and periostitis in rat given adjuvant. Proc. Soc. Exp. Biol. Med. **91**:95–101.
21. **Pearson, C. M., and F. D. Wood.** 1963. Studies of arthritis and other lesions induced in rats by the injection of mycobacterial adjuvant. VII. Pathologic details of the arthritis and spondylitis. Am. J. Pathol. **42**:73–95.
22. **Pearson, C. M., and F. D. Wood.** 1964. Passive transfer of adjuvant arthritis by lymph node or spleen cells. J. Exp. Med. **120**:547–560.
23. **Quagliata, F., and J. M. Phillips-Quagliata.** 1972. Competence of thoracic duct cells in the transfer of adjuvant disease and delayed hypersensitivity. Evidence that mycobacterial components are required for the successful transfer of the disease. Cell. Immunol. **3**: 78–87.
24. **Sato, V. L., S. D. Waksal, and L. A. Herzenberg.** 1976. Identification and separation of pre T-cells from nu/nu mice. Differentiation by preculture with thymic reticuloepithelial cells. Cell. Immunol. **24**:173–185.
25. **Scheid, M. P., G. Goldstein, and A. Boyse.** 1975. Differentiation of T cells in nude mice. Science **19**:1211–1213.
26. **Steffen, C., and G. Wick.** 1971. Delayed hypersensitivity reaction to collagen in rats with adjuvant-induced arthritis. Z. Immunitaetsforsch. Exp. Ther. **141**:169–180.
27. **Tanaka, A., S. Nagao, R. Saito, S. Kotani, S. Kusumoto, and T. Shiba.** 1977. Correlation of stereochemically specific structure in muramyl dipeptide between macrophage activation and adjuvant activity. Biochem. Biophys. Res. Commun. **77**:621–627.
28. **Unanue, E. R., and B. Benacerraf.** 1973. Immunologic events in experimental hypersensitivity granuloma. Am. J. Pathol. **71**:349–364.
29. **Vos, J. G., J. G. Kreeftenberg, B. C. Kruijt, W. Kuruizinga, and P. Streerenberg.** 1980. The athymic nude rat. II. Immunological characteristics. Clin. Immunol. Immunopathol. **15**:229–237.
30. **Waksman, B. H., C. M. Pearson, and J. T. Sharp.** 1960. Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant. II. Evidence that the disease is a disseminated immunologic response to exogenous antigen. J. Immunol. **85**:403–417.
31. **Whitehouse, D. J., M. W. Whitehouse, and C. M. Pearson.** 1969. Passive transfer of adjuvant-induced arthritis and allergic encephalomyelitis in rats using thoracic duct lymphocytes. Nature (London) **224**:1322–1324.