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Chronic Proliferative Arthritis of Mice Induced by *Mycoplasma arthritidis*: Demonstration of a Cell-Mediated Immune Response to Mycoplasma Antigens In Vitro

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Lymphocytes taken from mice chronically infected with *Mycoplasma arthritidis* exhibited a significant blastogenic response as measured by [^{3}H]thymidine uptake when exposed in vitro to *M. arthritidis* antigens. The lymphocytes taken from 9 of 12 control mice of similar age exhibited an inhibition of [^{3}H]thymidine uptake when exposed to *M. arthritidis* antigens.

Mycoplasma arthritidis induces a chronic proliferative arthritis of mice which both clinically and histologically closely resembles human rheumatoid arthritis (6, 7). Rodents appear to be defective in their antibody responses to *M. arthritidis* (3, 5) and in addition the organisms are not readily ingested by phagocytic cells (4, 14). *M. arthritidis* has been reported to suppress the humoral antibody responses of rats (12) and rabbits (2) to antigenic stimuli and the mitogenic responses of rat lymphocytes to phytohemagglutinin (PHA) after in vivo injection (12). Furthermore, the transformation of human lymphocytes by PHA is inhibited in vitro in the presence of *M. arthritidis* (10, 13, 15). The inhibition of mitosis observed appears to be related to arginine depletion of the medium (1, 10). Because of these observations and the possibility that chronic rheumatoid arthritis may be due to a delayed hypersensitivity response to a microbial antigen (11), we report here the cell-mediated immune responses of mice chronically infected with *M. arthritidis*.

Details of the cultivation of mycoplasmas, the preparation of mycoplasma antigens, and the scoring of mice for arthritis have been reported elsewhere (3, 6, 7). Female Swiss Webster mice 7 to 8 weeks of age were injected intravenously with 7.4×10^8 to 10^9 colony-forming units of *M. arthritidis* strain 158 P10 P9 and were sacrificed after 4.5 to 10.5 months. Control mice consisted of uninjected animals of similar age or mice previously injected intravenously with mycoplasma broth. The lymphocyte transformation technique described by Colley (8) was modified as follows: nodes and spleens were pooled for each mouse and were excised in RPMI 1640 medium with glutamine (no. 12-702, Microbiological Associates, Bethesda, Md.) containing 10 U of preservative-free heparin per ml. After the initial wash, erythrocytes were lysed in 3 ml of 0.83% NH_4Cl, centrifuged at $200 \times g$, and washed a second time. Two milliliters of lymphocyte suspension (1.5×10^6 to 2×10^6 cells/ml) were distributed into plastic disposable tubes (16 by 125 mm) and the following were added to triplicate tubes: (i) 10 μg of purified PHA (Burroughs Wellcome) or (ii) 2.5 μg and/or 10 μg of *M. arthritidis* protein antigen or (iii) lymphocytes alone. The tubes were pulsed for 24 h with 1 μCi of [^{3}H]thymidine (specific activity: 20 Ci/mmol) after an initial 48 or 72 h of incubation in 5% CO_2 at 37 C. Lymphocytes were harvested as described by Colley and DeWitt (9) except that NCS (Amersham-Searle) was used as solubilizer and spectrafluor (Amersham-Searle) as scintillation fluid. The uptake of [^{3}H]thymidine was measured on a Nuclear Chicago scintillation counter 720 series. Data were converted to disintegrations per minute by using the Channels Ratio method of quench correction (Packard Technical Bulletin, December 1965, no. 15) and were expressed as the mean disintegrations per minute observed in the triplicate cultures. Blastogenic indices were calculated from the ratio of disintegrations per minute in the presence of mitogen or antigen to disintegrations in lymphocyte cultures without mitogen or antigen.

The results are summarized in Tables 1 and 2. Mycoplasmas were not isolated from any of the control mice or from mice which had resolved their arthritis. Mycoplasmas were isolated from seven out of nine mice which exhibited active arthritis. Mice previously injected with *M. arthritidis* exhibited complement fixation antibody titers at sacrifice of 1:80 to 1:1280

TABLE 1. *Blastogenic responses of normal mouse lymphocytes to PHA and M. arthritidis antigens*

Mouse no.	Material injected	Age in months	PHA response			*M. arthritidis* antigen response				
			No mitogen	10 μg of PHA	Index	No antigen	2.5 μg of antigen	Index	10 μg of antigen	Index
1[a]		6	1,504	548,310	364.6	1,504	851	0.6	308	0.2
2[a]		8	574	131,389	228.9	574	NT[b]	NT	711	1.2
3[a]		8	2,567	370,279	144.2	2,567	13,620	5.3	11,127	4.3
4	Broth	6	590	73,700	124.9	2,097	NT	NT	693	0.3
5[a]		8	1,989	208,644	104.9	1,989	611	0.3	NT	NT
6[a]		10	2,943	250,493	85.1	2,943	NT	NT	2,357	0.8
7[a]		10	3,518	228,440	64.9	3,518	NT	NT	900	0.3
8[a]		8	1,191	118,798	99.7	1,191	1,106	0.9	NT	NT
9	Broth	6	1,458	59,914	41.1	1,488	NT	NT	1,620	1.1
10[a]		8	11,560	446,657	38.6	11,560	12,056	1.0	5,651	0.5
11	Broth	6	2,301	79,762	33.8	2,173	NT	NT	1,473	0.7
12[a]		8	16,189	491,878	30.4	16,189	12,975	0.8	4,514	0.3
Mean			3,865	250,688	113.4[c]	3,982	6,869	1.5[c]	2,914	1.0[c]

[a] [^{3}H]thymidine added to all tubes after 48 h of incubation.
[b] NT, Not tested.
[c] Mean calculated from indices of individual mice.

TABLE 2. *Blastogenic responses of lymphocytes taken from mice infected with M. arthritidis*

Mouse no.	Months post-infection	Arthritis score	Mycoplasmas isolated from:		PHA response			*M. arthritidis* antigen response				
			Joints	Nodes and spleen	No mitogen	10 μg of PHA	Index	No antigen	2.5 μg of antigen	Index	10 μg of antigen	Index
1	5	2	3/4	0	3,639	602,590	165.6	885	NT[a]	NT	13,181	14.9
2	4	0	0/4	0	1,712	231,190	135.0	2,247	NT	NT	11,948	5.3
3[b]	6.5	4	3/4	0	4,100	441,491	107.7	4,100	31,402	7.7	24,182	5.9
4	4	0	0/4	0	726	75,235	103.6	1,192	NT	NT	3,627	3.0
5	10.5	1	NT	NT	2,961	266,731	90.1	2,361	NT	NT	27,977	11.9
6	4.5	2	0/4	0	870	64,596	74.3	480	NT	NT	1,159	2.4
7	4.5	0	0/4	0	3,285	221,324	67.4	659	NT	NT	2,832	4.3
8	4.5	0	0/4	0	4,610	192,229	41.7	442	NT	NT	2,443	5.5
9	5	5	3/4	0	5,359	203,585	38.0	2,171	NT	NT	6,088	2.8
10[b]	6.5	3	3/4	+	5,178	180,000	34.8	5,178	7,074	1.4	19,403	3.8
11	4.5	0	0/4	0	1,007	31,973	31.8	514	NT	NT	825	1.6
12	4.5	2	0/4	0	482	11,241	23.3	2,621	NT	NT	1,294	0.5
13[b]	9.5	6	1/4	0	12,486	274,439	22.0	12,486	31,637	2.5	NT	NT
14	4.5	3	1/4	+	665	9,694	14.6	356	NT	NT	433	1.2
15	5	2	3/4	0	4,128	53,464	13.0	1,058	NT	NT	1,883	1.8
16	10.5	2	NT	NT	6,723	79,542	11.8	3,730	NT	NT	18,472	5.0
Mean					3,620	183,707	60.9[c]	2,530	13,951	3.9	9,049[c]	4.7[c]

[a] NT, Not tested.
[b] [^{3}H]thymidine added to all tubes after 48 h of incubation.
[c] Mean calculated from indices of individual mice.

against *M. arthritidis* antigens. None of the control mice exhibited complement fixation antibody titers greater than 1:10. Lymphocytes taken from both control and infected mice responded to PHA. The somewhat higher mean index obtained with control mice (113.4, Table 1) as compared with infected mice (60.9, Table 2) was due in part to two control mice (no. 1 and 2) that exhibited unusually high indices. Using the Welch *t* test, statistical analysis indicated a low level of significance between the groups ($P = 0.103$). Due to the variability in background [^{3}H]thymidine uptake, the results were reexamined by comparing directly disintegrations per minute of control and sensitized lymphocytes exposed to PHA. No significance between the two groups was apparent ($P = 0.300$). *M. arthritidis* antigen, particularly at a concentra-

tion of 10 μg/tube, inhibited the uptake of [^{3}H]-thymidine by lymphocytes from nine of 12 normal mice. The lymphocytes from only one of 12 normal mice exhibited significant enhanced activity in response to *M. arthritidis* antigen. The mean index of all normal lymphocytes exposed to 10 μg of antigen was 1.0.

Lymphocytes taken from mice previously infected with *M. arthritidis* exhibited a blastogenic response to *M. arthritidis* antigens, i.e., mean indices were 3.9 using 2.5 μg of antigen and 4.7 using 10 μg of antigen as compared with control cultures containing no antigen. Only one of 16 previously infected mice possessed lymphocytes which exhibited a decreased uptake of [^{3}H]thymidine in the presence of *M. arthritidis* antigens. Statistical analysis of all results revealed that the values observed in arthritic mice as compared with controls were highly significant by comparing either indices ($P = 0.002$) or disintegrations per minute ($P = 0.012$). There was no correlation between the severity of arthritis and blastogenesis.

These results suggest a consistent but low in vitro blastogenic response of sensitized lymphocytes to *M. arthritidis* antigen. Our observations that *M. arthritidis* inhibits the metabolism of normal lymphocytes has been observed by other investigators. We have also shown that the same preparation of *M. arthritidis* as used in the present study also inhibited the uptake of [^{3}H]thymidine by human lymphocytes (unpublished observations). Since the positive transformations observed with this antigen in the presence of sensitized lymphocytes reflect the outcome of both inhibitory and stimulatory antigen components, it is clear that the true degree of sensitization of arthritic mice to *M. arthritidis* may be masked. Whether *M. arthritidis* inhibits blastogenesis in vivo remains to be determined; however it has been reported that this organism can lead to T cell depletion after injection into mice (R. J. Eckner, T. Han, and V. Kumar, Fed. Proc. **33**:769, 1974). These observations may explain the failure to correlate degree of lymphocyte transformation with severity of arthritis since mice exhibiting arthritis harbored mycoplasmas more frequently than those which did not.

The detection of a cell-mediated immune reaction in vitro to *M. arthritidis* appears not to be restricted to mice since preliminary experiments have shown that two rats infected 6 weeks previously with *M. arthritidis* exhibited blastogenic indices of 3.1 and 8.1, respectively, when their lymphocytes were exposed to homologous antigen.

Further studies are required to define whether the reactions observed are B or T cell-dependent and whether cell-mediated immune reactions play a role in the development of chronic inflammation.

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