Arthritis of mice induced by *Mycoplasma arthritidis*

Humoral antibody and lymphocyte responses of CBA mice

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Cole, B. C., Golightly-Rowland, L., and Ward, J. R. (1976). *Annals of the Rheumatic Diseases*, 35, 14–22. Arthritis of mice induced by *Mycoplasma arthritidis*. Humoral antibody and lymphocyte responses of CBA mice. Peak arthritis occurred 7 days after intravenous injection of CBA mice with *Mycoplasma arthritidis* and persisted in some animals for 84 days. A marked leucocytosis was apparent for the first 21 days. Complement-fixing antibodies reached a peak 14 days after injection of the organisms and persisted at high levels for 84 days. Metabolic-inhibiting and mycoplasmacidal antibodies were present but at much lower titres.

Lymphocytes taken from mice 7, 14, 21, and 35 days after injection of *M. arthritidis* exhibited an enhanced uptake of ^3H thymidine in the absence of added antigen as compared with lymphocytes from control mice. An immune response as indicated by enhanced uptake of ^3H thymidine by sensitized lymphocytes exposed to antigen was apparent at all stages of the disease process. Lymphocyte transformation was maximal at 3 days and minimal at 21 days. *M. arthritidis* infection *in vivo* had no effect on the ability of lymphocytes to transform in response to either phytohaemagglutinin or lipopolysaccharide.

No correlation was apparent between severity of arthritis and either humoral or cell-mediated immune responses of the mice. Whereas persisting arthritis correlated with persisting viable mycoplasmas, challenge of the joints of convalescent mice with viable mycoplasmas did not precipitate or enhance the arthritis.

Mycoplasma arthritidis induces a chronic arthritis of Swiss Webster mice characterized by periods of remission and exacerbation and a histological picture closely resembling that of human rheumatoid arthritis (Cole and others, 1971b). Whereas the organisms persist in the joints for at least 217 days, injected animals exhibit some immunity to intravenous challenge with viable organisms (Cole and others, 1971a). While complement-fixing antibodies persist in injected mice, metabolic-inhibiting antibodies are produced in low titre only and are not detected in the chronic phase of the disease (Cole and others, 1971a). Antibodies, cidal toward resting cells only, are rapidly produced in high titres (Cole and Ward, 1973b). The apparent absence of antibodies which could eliminate multiplying organisms *in vivo* correlates with the inability to transfer im-

munity to normal mice by administration of convalescent serum (Cole and others, 1971a). In contrast the injection of rabbit anti-*M. arthritidis* serum which contains neutralizing antibodies into normal mice does confer protection.

Experiments on the cellular defences of the host show that macrophages are unable to phagocytose *M. arthritidis* even in the presence of convalescent serum, thus suggesting that mice do not produce opsonizing antibodies against the invading organisms. Whereas rats are more efficient than mice in handling infections by *M. arthritidis*, similar deficiencies in the immune responses are apparent (Cole and others, 1969; Cole and Ward, 1973a). In the case of rats an antigenic component common to host and parasite may explain this observation (Cahill and others, 1971).

Preliminary experiments have shown that lymphocytes taken from mice chronically infected for $4\frac{1}{2}$ to $10\frac{1}{2}$ months with *M. arthritidis* exhibit a signi-

ficant blastogenic response when exposed to *M. arthritidis* antigen (Cole, Golightly-Rowland, and Ward, 1975). The lymphocytes of control mice exhibit a decrease in uptake of ^3H thymidine in the presence of *M. arthritidis* antigen, thus confirming the previous observations of the inhibitory effect on mitosis by this organism (Barile and Levinthal, 1968; Copperman and Morton, 1966; Simberkoff, Thorbecke, and Thomas, 1969).

The present study was undertaken to document the development of the lymphocyte transformation reaction and to compare this with humoral antibody and severity of arthritis. The claimed immunosuppressive properties of *M. arthritidis* (Bergquist, Lau, and Winter, 1974; Eckner, Han, and Kumar, 1974; Simberkoff and others, 1969) were also investigated by determining the effects of this organism on the mitogenic capabilities of phytohaemagglutinin, a T-cell mitogen (Janossy and Greaves, 1971), and lipopolysaccharide, a B-cell mitogen (Andersson, Sjöberg, and Möller, 1972).

Materials and methods

CULTIVATION OF MYCOPLASMAS

M. arthritidis strain 158 P10 P9 (Cole and others, 1971b) was cultured in Difco mycoplasma broth or agar supplemented to final concentrations of 10% sterile horse serum, 1% fresh yeast extract, 0.1% L-arginine HCl (Eastman), and 1000 units/ml penicillin (Chanock, Hayflick, and Barile, 1962; Hayflick, 1965). The preparation and concentration of mycoplasma cultures used for injection and antigen production were as previously described (Golightly-Rowland and others, 1970). Antigen suspensions were subjected to ultrasonic vibration in two-minute intervals until no viable organisms remained when tested on mycoplasma agar, and protein concentrations of the suspensions were determined by the Lowry procedure (Lowry and others, 1951).

INDUCTION OF ARTHRITIS AND AUTOPSY

A concentrated culture of *M. arthritidis* 158 P10 P9 was diluted to contain 2×10^9 colony-forming units per 0.2 ml and injected intravenously into 6- to 8-week-old female CBA mice. Control mice were injected with 0.2 ml mycoplasma broth. All haematological, serological, lymphocyte, and cultural studies were performed on the same mice.

Four control mice and eight mice injected with *M. arthritidis* were sacrificed after 3, 7, 14, 21, 35, 56, and 84 days, each animal having previously been scored for arthritis by subjectively measuring joint swelling. Ankles, wrists, metatarsal joints, metacarpal joints and digits were graded on a 0-4 scale and totaled. Then each animal was bled from the retro-orbital venous plexus using a heparinized 0.05 ml capillary tube. A viable mycoplasma count was performed on half the blood, the other half being used for differential and total white blood count. Blood was drained from the animals, by cardiac puncture, was allowed to clot, and serum separated for serological tests.

Spleens and lymph nodes were transferred to Petri dishes containing 5 ml RPMI 1640 media (Microbiological Associates, Albany, Calif.) for lymphocyte stimulation studies.

ISOLATION OF MYCOPLASMA

Isolation of mycoplasma from wrist and ankle joints was attempted by removing hind and forepaws and exposing the tibiotarsal and radiocarpal joints and streaking the exposed surface on an agar plate. Mycoplasma isolations from spleens and nodes were also attempted by streaking 0.5 ml of the minced tissue suspensions used for blastogenesis on mycoplasma agar plates. After 7 days all isolation plates were observed for the presence of mycoplasma.

TISSUE CULTURE MEDIA

The tissue culture medium used throughout the experiments was RPMI 1640 with L-glutamine (Microbiological Associates), containing 100 units/ml penicillin-streptomycin mixture (Microbiological Associates). Culture tubes were supplemented with 2% heat inactivated (56° for 30 min) human serum. Tubes of the serum were stored at -20°C and thawed for each experiment.

PREPARATION OF CELL CULTURES

Colley's (1971) method was modified as follows. Spleens and nodes were placed in separate Petri dishes containing 5 ml RPMI 1640 medium. Tissues were teased gently with a scalpel blade. Suspensions were then filtered through a 60-mesh stainless steel screen and centrifuged for 5 min at 200 g. 3 ml of prewarmed (37°C) 0.83% $\text{NH}_4\text{-Cl-Tris}$ buffered to pH 7.2 was added to lyse red blood cells (Boyle, 1968). After gentle mixing the suspensions were centrifuged at 200 g for 5 minutes. Cells were resuspended in 5 ml of fresh medium and recentrifuged at 200 g. The number of viable cells was determined by trypan blue exclusion (Turk, Glade, and Chessin, 1969). Cells were resuspended to a final concentration of 1.5×10^6 /ml complete RPMI 1640 medium. No culture with more than 22% dead cells was used.

Node and spleen suspensions of each mouse were distributed as follows. 2 ml of spleen culture were dispensed into each of six 16 × 125 mm plastic disposable tubes (Falcon 3033). 20 µg lipopolysaccharide B (*Escherichia coli* 055; B5, Difco) in 0.2 ml was added to three of the tubes. The remaining spleen culture was pooled with the lymph node culture and dispensed into nine tubes. Pooled lymphocyte cultures contained from 10-30% node cells. Triplicate tubes of the pool were stimulated with 0.2 ml mycoplasma antigen containing 5 µg protein or 0.2 ml of a 1:50 dilution of PHA (reagent grade #HA15, Burroughs Wellcome, Tuckahoe, N.Y.). Three control tubes were also included. Stimulants used for blastogenesis were pretitrated for optimal stimulation. All tubes were incubated at 37°C in an atmosphere of 5% CO_2 and air for 72 hours. 24 hours before harvesting 1.0 µCi tritiated thymidine (specific activity 19 Ci/mmol, Amersham Searle Corp., Arlington Heights, Ill.) was added to each tube.

MEASUREMENT OF BLASTOGENESIS

Lymphocyte cultures were harvested according to Colley and DeWitt (1969). Cultures were transferred to 12 ml

glass conical centrifuge tubes and washed twice with cold PBS at pH 7.2. Tubes were centrifuged at 200 g for 5 minutes after each wash. Cultures were then treated with 0.5 ml 1 N NaOH and incubated for 10 minutes at 56°C after which cells were treated twice with 2 ml cold 5% trichloroacetic acid (centrifuged for 5 minutes at 1250 × g each time). After the second trichloroacetic acid treatment precipitates were dissolved by adding 0.5 ml of NCS tissue solubilizer (Amersham/Searle) and agitating with a vortex mixer. 10 ml of scintillation fluid (42 ml Liquiflour, New England Nuclear, Los Angeles, Calif.) per litre scintillation grade toluene (Matheson Coleman and Bell, Los Angeles, Calif.) was added to each tube. After thorough mixing the tube contents were transferred to scintillation vials (Packard #600175, Downers Grove, Ill.) and allowed to dark adapt for 5 hours. Samples were counted in a Beckman LS-233 liquid scintillation counter with an automatic external standard (Beckman Instrument Inc., Fullerton, Calif.). Counts were converted to disintegrations per minute (dpm) by employing a standard curve relating per cent. efficiency to the external standard which allows for a quench correction. Most samples were counted at 35–40% efficiency. The dpm in the triplicate lymphocyte cultures were averaged.

SEROLOGICAL INVESTIGATIONS

All mouse sera tested for antibody titres against *M. arthritidis* were heat inactivated at 56°C for 30 minutes. The microtechnique of Purcell and others (1966), was used to determine metabolic inhibiting antibody in which inhibition of the metabolism of arginine was used as the indicator. Complement-fixing antibody was detected by the micromethod of Casey (1965). The same antigen used for lymphocyte stimulation was used in the complement-fixing test. Mycoplasmacidal antibodies were detected according to Cole and Ward (1973b). The mean antibody titres for control mice and infected mice were reported for each time period.

RESISTANCE OF CONVALESCENT MICE TO JOINT CHALLENGE BY *M. ARTHRITIDIS*

Swiss Webster female mice, 7 weeks old, were injected intravenously with 7.4×10^8 colony-forming units (CFU) *M. arthritidis* strain 158 P10 P9. After 115 days mice with resolved arthritis were sorted into groups of 6–10 mice each and were challenged in the right ankle joint with approximately 0.05 ml of (1) 10^6 CFU *M. arthritidis* in saline, (2) 10^4 CFU *M. arthritidis* in saline, (3) *M. arthritidis* membrane suspension, (4) *M. arthritidis*

membrane diluted 10^{-3} , (5) saline alone. Animals injected 115 days previously with mycoplasma broth were similarly injected and served as controls. The mice were scored for arthritis at intervals up to 287 days.

Crude membranes were prepared from a mycoplasma suspension originally containing 2×10^{10} CFU/ml. The cells were washed 3 times by centrifugation at 27000 × g, resuspended in distilled water and sonified for 5 minutes using a Branson sonifier. Unbroken cells were removed by low speed centrifugation (8000 × g) and the membranes were washed 3 times in saline by centrifugation at 37000 × g. The membranes were resuspended to the same volume as the original mycoplasma suspension.

Results

Table I shows the number of mice used in these studies and development of arthritis. Maximum arthritis occurred at 7 days and some animals still exhibited joint inflammation 12 weeks after injection of the organisms. The CBA mice used in this study appeared to be less susceptible than the Swiss Webster mice used in previous work (Cole and others, 1971b). No deaths were recorded.

Mycoplasmas could be isolated from most mice up to 5 weeks, and persisted in one animal for 12 weeks (Table I). Blood, spleen, and nodes were usually free of mycoplasmas after 7 days but occasionally mycoplasmas were isolated at a later time. Organisms could usually be cultured from joints up to 35 days after injection. After the initial acute phase of the disease, most animals which retained mycoplasmas in the joints exhibited arthritis. Not only did a higher proportion of arthritic mice harbour mycoplasmas, but there was also a correlation between numbers of mycoplasmas isolated and arthritis score. However, further analysis of the data indicated that 11% of arthritic joints were free of mycoplasmas. More exhaustive studies would probably decrease this number. Mycoplasmas could not be isolated from any of the control mice.

EFFECT ON LEUCOCYTE COUNTS

A marked rise in the total white cell count was apparent from 3 days to 21 days in mice which had been

Table I Isolation of mycoplasmas and development of arthritis

Days after injection	No. of infected mice	No. of control mice*	No. of infected mice from which mycoplasmas were cultured				% of mice with arthritis	Mean arthritis	
			Blood	Spleen	Nodes	Joints		Score	Range
3	7	4	7	6	5	7	100	2.7	1–5
7	9	7	3	2	2	8	100	4.0	1–10
14	8	4	0	1	0	5	62	1.4	0–6
21	8	4	0	0	0	3	75	1.8	0–6
35	7	4	1	0	0	4	71	1.6	0–4
56	6	4	0	0	0	0	50	0.5	0–1
84	8	4	1	0	0	0	25	0.8	0–5

* Blood, spleen, and nodes of all control mice were free of mycoplasmas. Joints were not routinely cultured from control animals.

FIG. 1 Total white blood cell counts in control mice (open bars) and in mice injected with 2×10^9 colony-forming units *M. arthritidis* (solid bars). Conversion: Traditional Units to SI—WBC: $1.0 \times 10^3/\text{mm}^3 \approx 1.0 \times 10^9/\text{l}$

FIG. 2 Changes in peripheral white blood cell types in mice injected with *M. arthritidis*. Polymorphonuclear leucocytes in control mice ○ and in infected mice ●. Lymphocytes in control mice △ and in infected mice ▲. Conversion: Traditional Units to SI—Leucocytes: $1.0 \times 10^3/\text{mm}^3 \approx 1.0 \times 10^9/\text{l}$

FIG. 3 Humoral antibody response of CBA mice to infection with *M. arthritidis*. Complement-fixing antibody ●, metabolic-inhibiting antibody ○, and mycoplasmacidal antibody ▲

injected with *M. arthritidis* (Fig. 1). The leucocyte count approached normal values at 35 and 56 days but was slightly raised 84 days after injection. Differential counts (Fig. 2) showed a marked early increase in polymorphonuclear leucocytes reaching a peak 7 days after injection of organisms and a lymphocytosis reaching a peak 14 days after injection of organisms. At 35 days the leucocyte population was similar in both control and infected mice; however, a slight increase in polymorphonuclear leucocytes was apparent at both the 56- and 84-day periods.

HUMORAL ANTIBODY RESPONSES

The humoral antibody responses of mice to *M. arthritidis* are summarized in Fig. 3. Complement-fixing antibody was first detected 7 days after injection of the organisms, reaching a peak after 14 days, and persisting in high titre throughout 84 days. Metabolic inhibiting antibody was not detected until 14 days. The titres were less than those observed with the complement-fixation test but persisted throughout the experiment. Mycoplasmacidal antibody appeared at 3 days and also persisted.

LYMPHOCYTE RESPONSES TO ANTIGEN

Due to possible variations in the sensitivity of assays performed on different days control mice and infected mice were included in each set of assays. Lymphocytes taken from control and infected mice were compared for their ability to incorporate ^3H thymidine in the absence of *M. arthritidis* antigen (dpm without antigen, Table II). No significant differences were apparent at 3, 56, and 84 days. However, from 7 to 35 days lymphocytes taken from infected mice exhibited an enhanced uptake of ^3H thymidine (maximum of 3.6 fold at 21 days) which was statistically significant at the 95% confidence level. Due to these enhanced background levels of ^3H thymidine uptake by sensitized lymphocytes, the results of blastogenic transformations in the presence of antigen are recorded as dpm observed in lymphocytes from infected mice as compared with dpm observed in lymphocytes from control mice.

In the present study $5 \mu\text{g}$ of *M. arthritidis* antigen was used for the blast transformations since it exhibited only a minimal inhibitory effect toward ^3H thymidine uptake by normal lymphocytes. However, a different antigen preparation, which was used for animals sacrificed at the 8- and 12-week periods, was found to be more inhibitory toward normal lymphocytes at the $5 \mu\text{g}/\text{tube}$ concentration.

The lymphocytes from mice previously injected with *M. arthritidis* showed a significant blastogenic transformation when exposed to homologous antigen. This response was present at all stages of the disease process and was statistically significant at the 95%

Table II *Blastogenic responses of lymphocytes taken from control and infected mice to M. arthritidis antigen*

Days after injection*	dpm observed in lymphocyte cultures					
	Without antigen (background levels)			With <i>M. arthritidis</i> antigen		
	Control mice	Infected mice	Ratio†	Control mice	Infected mice	Ratio†
3	2653	2403	0.9	2996	45655	15.2
7	3112	6720	2.2	6182	29853	4.8
14	1587	4575	2.9	580	10846	18.7
21	1551	5501	3.6	1140	10369	9.1
35	1668	5209	3.1	813	16010	19.7
56	27134	49899	1.8	5993	70850	11.8
84	14812	18365	1.2	2241	27024	12.1

* Control mice were injected with mycoplasma broth. Infected mice were injected with *M. arthritidis*.

† Ratio of dpm in lymphocytes from infected mice to dpm in lymphocytes from control mice.

confidence levels or greater at all times. Control lymphocytes taken from mice injected with mycoplasma broth did not transform significantly when exposed to *M. arthritidis* antigen; in fact an inhibitory effect was observed at days 14, 21, 35, 56, and 84. Despite the unusually high background levels of ³H thymidine incorporation at 56 and 84 days, and the inhibitory properties of the antigen preparation toward normal lymphocytes, a significant blastogenic response occurred with sensitized lymphocytes exposed to homologous antigen.

EFFECT OF *M. ARTHRITIDIS* INFECTION ON THE LYMPHOCYTE RESPONSE TO PHYTOHAEMAGGLUTININ (PHA) AND LIPOPOLYSACCHARIDE
These studies were undertaken to attempt to confirm reports that *M. arthritidis* infection *in vivo* can inhibit the blastogenic response of lymphocytes exposed to PHA (Eckner and others, 1974; Kaklaminis and Pavlatos, 1972). The effect on the B-cell mitogenic agent, bacterial lipopolysaccharide, was also investigated.

The lymphocytes taken from both control and infected mice transformed similarly when exposed

to PHA (Table III). Statistical analyses failed to detect a significant difference between the groups ($P > 0.1$). Examination of blastogenic indices, *i.e.* ratio of ³H thymidine in the presence of mitogen as compared to no mitogen, indicated mean values at various times of 68–376 for the control lymphocytes and 52–293 for the lymphocytes from infected mice. Similarly, the ability of lymphocytes to respond to bacterial lipopolysaccharide was not significantly impaired ($P > 0.1$) by previous *in vivo* infection by *M. arthritidis* (Table III).

CORRELATION BETWEEN SEVERITY OF ARTHRITIS, IMMUNE RESPONSES, AND PRESENCE OF ORGANISMS

An attempt was made to correlate persisting arthritis with immune responses and presence of viable organisms. All mice which had been injected 14 or more days previously with *M. arthritidis* were distributed into 3 groups depending upon severity of arthritis. The results are summarized in Table IV. Although no statistically significant correlation between the humoral antibody or lymphocyte response to antigen was found, there was an associa-

Table III *Effect of M. arthritidis infection in vivo on the mitogenic responses of spleen lymphocytes to phytohaemagglutinin (PHA) and lipopolysaccharide*

Days after injection	dpm $\times 10^3$ in lymphocyte cultures ^a exposed to mitogen					
	PHA exposure			Lipopolysaccharide exposure		
	Control mice	Infected mice	Ratio*	Control mice	Infected mice	Ratio*
3	528.1	534.6	1.0	75.1	59.1	0.8
7	1105.7	452.2	0.4	108.4	69.7	0.6
14	359.6	665.8	1.9	194.1	217.9	1.1
21	349.9	247.7	0.7	NT	NT	NT
35	248.9	428.0	1.7	214.6	288.0	1.3
56	1587.6	1727.3	1.1	117.9	103.7	0.9
84	613.9	769.2	1.3	77.5	84.2	1.1

* Ratio of dpm in lymphocytes from infected mice exposed to mitogen as compared to dpm in control lymphocytes exposed to mitogen.

Table IV Relationship between arthritis, immune response, and persistence of viable organisms

No. of mice*	Arthritis score	Reciprocal of mean antibody titres			Lymphocyte response to antigen (Mean dpm $\times 10^3$)	% of mice harbouring mycoplasmas
		CF	MI	Cidal		
15	0	568	61	18	24	6.3
15	1-2	720	69	20	33	33.3
7	>2	1142	37	13	14	85.7

* Mice were injected 14 or more days previously with *M. arthritidis*.

Table V Effect of intra-articular injection of *M. arthritidis** in control and convalescent mice

Material injected	No. of mice	Mean arthritis scores† after the following days:			
		3	10	28	49
Control mice					
Viable <i>M. arthritidis</i> 10 ⁶ /mouse	7	1.4	5.4	3.6	1.7
<i>M. arthritidis</i> membranes	7	0	0	0	0
Saline	7	0	0	0	0
Convalescent mice					
Viable <i>M. arthritidis</i> 10 ⁶ /mouse	10	0	0	0	0
<i>M. arthritidis</i> membranes	6	0.1	0	0	0.1
Saline	10	0	0	0	0

* Control mice received intravenous mycoplasma broth 115 days before challenge and convalescent mice received intravenous *M. arthritidis* before challenge.

† Only arthritis occurring in the injected limb is reported here.

tion between persisting arthritis and the presence of culturable mycoplasmas.

RESISTANCE OF CONVALESCENT MICE TO JOINT CHALLENGE BY *M. ARTHRITIDIS*

These studies were undertaken to explore further the possible roles of viable organisms or cell-mediated immune responses in development of chronic arthritis. Convalescent mice were injected in one joint with viable *M. arthritidis*, *M. arthritidis* membranes, or saline. Control mice previously injected with mycoplasma broth were similarly treated.

Control mice developed severe arthritis in the injected limb with either 10⁶ or 10⁴ CFU *M. arthritidis* (Table V, Fig. 4). Neither *M. arthritidis* mem-

branes nor saline induced a detectable inflammatory response. The course of arthritis produced by joint injection of *M. arthritidis* paralleled that seen earlier (Cole and others, 1971b) resulting from intravenous injection in that a resurgence in inflammation occurred 15 to 23 weeks after injection (Fig. 4). It is of interest in the present study that no arthritis occurred in limbs which were not injected.

Convalescent mice were immune to challenge with viable *M. arthritidis*. During the first 7 weeks a few mice developed minimal arthritis after the injection of 10⁶ CFU *M. arthritidis* and *M. arthritidis* membranes but this rarely occurred in the injected joint. The minimal arthritis seen could be due to the spontaneous exacerbation of arthritis which is characteristic of this disease. The development of arthritis in one mouse receiving saline supports this view. Thus, continued examination of these mice through 41 weeks indicated a slightly enhanced arthritis in all groups. There was no relationship between joints injected and joints which developed arthritis.

Discussion

The Mycoplasmatales cause a wide variety of respiratory and arthritic diseases in animals and hence may be potential mediators of human rheumatoid arthritis. The chronicity of mouse arthritis induced by *M. arthritidis* indicates that this is a

FIG. 4 Development of chronic arthritis in mice after intra-articular injection of the right ankle with 10⁶ colony-forming units ● or 10⁴ CFU ○ of *M. arthritidis*

suitable model for showing the mechanisms of inflammation. A commonly held theory is that human RA may be due to a cell-mediated immune reaction against an unknown infectious agent or combination of the agent with self antigens (Ford, 1963; Lawrence, 1959). Cell-mediated immune responses to mycoplasmas in infections have only recently received attention. However, evidence has been presented that in both *M. pneumoniae* and *M. pulmonis* (Denny, Taylor-Robinson, and Allison, 1972; Fernald, 1970; Fernald and Clyde, 1974; Taylor and Taylor-Robinson, 1974) respiratory disease a cell-mediated immune response contributes to the development of the lesion. In a brief report we described evidence of a cell-mediated immune response to *M. arthritis* in Swiss Webster mice chronically infected with *M. arthritis* for 4 to 10½ months. In the present study lymphocytes taken from CBA mice infected with *M. arthritis* transformed significantly at all stages of the disease when exposed to homologous antigen *in vitro*. However, there was no relationship between severity of arthritis and degree of lymphocyte transformation, a reaction which usually correlates with delayed hypersensitivity (Dutton, 1966; Oppenheim, 1968). Similar observations were made previously using Swiss Webster mice chronically infected with *M. arthritis* (Cole and others, 1975). In both studies, however, persisting arthritis was associated with the presence of viable mycoplasmas in the joints. Thus, a cell-mediated immune reaction to persisting antigen could contribute to the disease process. Evidence against this hypothesis is the observation that mice sensitized to *M. arthritis* did not develop an inflammatory reaction after intra-articular challenge with viable or nonviable antigen.

Examination of individual animals also failed to show a correlation between humoral antibody titres and persisting arthritis. The severity of arthritis obtained in CBA mice was noticeably less than that previously obtained in Swiss Webster mice (Cole and others, 1971b), perhaps correlating with the higher levels of metabolic-inhibiting (MI) antibody produced by CBA mice in response to *M. arthritis* infection. However, mycoplasmas could still be isolated from some animals which exhibited MI antibody titres. Evidence that the humoral antibody response of the host has at least some effect on the development of the disease is apparent by the observation that avirulent strains of *M. arthritis* induce higher levels of MI antibody in Swiss Webster mice than do virulent strains (unpublished observations).

Since challenge of convalescent Swiss Webster mice intravenously (Cole and others, 1971a) or intra-articularly with *M. arthritis* does not result in inflammation, some host defences are clearly controlling the infectious process. Cellular immunity may play a major role since lymphocyte transforma-

tion was present at all stages of the disease process. In addition, we have previously failed to transfer immunity to *M. arthritis* in Swiss Webster mice by passive administration of convalescent serum (Cole and others, 1971a). Similarly, we have failed to detect opsonizing antibodies in convalescent Swiss-Webster mouse sera which might promote phagocytosis of the invading organisms (Cole and Ward, 1973a).

The ability of *M. arthritis* to inhibit *in vitro* the response of lymphocytes to phytohaemagglutinin (PHA) due to arginine depletion is well documented (Barile and Levinthal, 1968; Copperman and Morton 1966; Simberkoff and others, 1969), but a number of recent reports suggest that *M. arthritis* may also be immunosuppressive *in vivo*. Thus Kaklaminis and Pavlatos (1972) reported that *M. arthritis* infection *in vivo* resulted in a decreased ability of rat node lymphocytes to respond to PHA as evidenced by a decreased presence of blast type cells upon microscopical observation. Eckner and others (1974) reported that thymocytes taken from mice infected with *M. arthritis* did not respond to the mitogenic effects of PHA. Our studies are at variance with the observations. Thus *M. arthritis* infection *in vivo* was found to have no effect on the response of lymphocytes to either PHA (a T-cell mitogen, Kaklaminis and Pavlatos, 1972) or bacterial lipopolysaccharide (a B-cell mitogen, Andersson and others, 1972). These results are consistent with our observations that *M. arthritis* induces both a cell-mediated and humoral response in mice. *M. arthritis* has also been reported to suppress humoral antibody production to other antigens (Bergquist and others, 1974; Kaklaminis and Pavlatos, 1972). Whether antigenic competition or some other mechanism is operative remains to be determined.

Whereas the exact mechanisms of chronic mouse arthritis remain elusive it is likely that persisting mycoplasmas may play a role in the inflammatory process. The occurrence of identical or similar antigens common to parasite and host could provide a mechanism for the development of autoimmunity to joint tissue (Cahill and others, 1971), this leading to a chronic arthritic process. However, it appears unlikely that a humoral or cell-mediated response to normal tissue is the explanation for chronic mouse arthritis, since normal mice injected intra-articularly with *M. arthritis* develop a chronic arthritis only in the injected joint. However, persisting mycoplasmas might damage host tissue thus providing the stimulus for an autoimmune response.

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