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CELL-MEDIATED IMMUNE RESPONSE FOLLOWING *MYCOPLASMA PNEUMONIAE* INFECTION IN MAN

I. LYMPHOCYTE STIMULATION

G. BIBERFELD,* P. BIBERFELD AND G. STERNER

National Bacteriological Laboratory, Departments of Immunology and Pathology, Karolinska Institutet, and Department of Infectious Diseases, Danderyd Hospital, Stockholm, Sweden

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SUMMARY

Lymphocytes from seventeen cases of recent *Mycoplasma pneumoniae* infection, thirteen cases of previous infection and ten healthy subjects lacking antibodies to *M. pneumoniae* were cultured in the presence of various antigen preparations of *M. pneumoniae*. Lymphocyte stimulation was measured by the uptake of [^{14}C]thymidine and by counting of morphologically transformed cells. The lymphocyte response to a sonicated concentrate of *M. pneumoniae* organisms and to a membrane fraction was significantly higher in recently infected patients than in healthy controls. However, nine out of ten serologically negative controls showed some degree of lymphocyte reactivity to *M. pneumoniae*. A lipid fraction of *M. pneumoniae*, which had a high antigen titre in the complement fixation test, induced no appreciable stimulation of lymphocytes from infected cases or control subjects.

Lymphocyte responsiveness to sonicated *M. pneumoniae* antigen was demonstrable up to 10 years after infection.

Among the cases with recent or previous *M. pneumoniae* infection there was no correlation between the degree of lymphocyte stimulation and the titres of antibodies to *M. pneumoniae*.

INTRODUCTION

M. pneumoniae is an important cause of lower-respiratory-tract illness (Grayston, Foy & Kenny, 1969) particularly in children and young adults. Infection with *M. pneumoniae* spreads extensively in closed populations, for instance in military camps, homes for children and in families (Grayston *et al.*, 1969). Efforts are therefore being made to develop an effective *M. pneumoniae* vaccine (Chanock, 1970). However, it is not known if humoral or

* Present address: The Stockholm County Council Central Microbiological Laboratory, Stockholm, Sweden.

Correspondence: Dr Gunnel Biberfeld, The National Bacteriological Laboratory, S-105 21 Stockholm, Sweden.

cell-mediated immunity is most important for protection against *M. pneumoniae* illness and accordingly which test should be used to measure protective immunity. Various tests are available for the demonstration of antibodies to *M. pneumoniae* (Purcell, Chanock & Taylor-Robinson, 1969). Studies in volunteers infected with *M. pneumoniae* showed that the presence of growth inhibiting antibody in serum was associated with lower rates of illness (Smith *et al.*, 1967). However, in hamsters experimentally infected with *M. pneumoniae* serum antibody levels did not correlate with protection against pneumonia (Fernald & Clyde, 1970). This and results of other immunopathological studies in infected hamsters suggested that cell-mediated immunity may be important for resistance to *M. pneumoniae* infection (Fernald, Clyde & Bienenstock, 1972).

The present and the following paper describe studies of the cell-mediated immune response following *M. pneumoniae* infection in man by the use of lymphocyte stimulation and leucocyte migration inhibition assays. The first paper deals with the results of lymphocyte stimulation tests. An account of part of this work has been given earlier (Biberfeld, 1972).

M. pneumoniae-induced lymphocyte stimulation *in vitro* has been demonstrated in volunteers experimentally infected with *M. pneumoniae* (Leventhal *et al.*, 1968). Recently Fernald (1972) presented the results of a study of lymphocyte stimulation in naturally infected cases.

MATERIALS AND METHODS

Subjects tested

The lymphocyte response to *M. pneumoniae* antigen was examined in thirty patients with recent or previous *M. pneumoniae* infection who had been treated at the department of infectious diseases of Danderyd hospital. Twenty-six of these cases had been hospitalized at the acute phase of illness. Twenty-eight cases had pneumonia as verified by X-ray and the other two cases had febrile bronchitis. The demonstration of a four-fold or greater rise in titre of complement-fixing (CF) and/or tetrazolium reduction inhibiting (TRI) (= growth inhibiting) antibodies in association with the illness or a CF antibody titre of $\geqslant 128$ together with cold agglutinins at titre $\geqslant 64$ was considered evidence of recent *M. pneumoniae* infection.

The lymphocyte stimulation test was also performed with lymphocytes from ten healthy blood donors who lacked CF (titre <4) and TRI antibodies (titre <2) to *M. pneumoniae.*

M. pneumoniae antigen

M. pneumoniae strain FH, obtained from Dr R. M. Chanock, NIH, Bethesda, was used for antigen preparation. *M. pneumoniae* organisms were grown on the surface (Somerson *et al.*, 1967) of glass bottles each containing 500 ml of broth medium. The medium (Hayflick, 1965) was composed of seven parts Difco PPLO broth, two parts unheated horse serum, one part 25% yeast extract, 1% glucose, 0·002% Phenol Red, 0·05 M of HEPES (Calbiochem) and penicillin 1000 units/ml. After incubation at 37°C for 4–5 days the broth was discarded, and the organisms attached to the glass surface were washed four times with phosphate-buffered saline (PBS) and then removed by shaking the bottle with sterile glass beads in PBS. The suspension of organisms was centrifuged at 15000 ***g*** for 30 min and then resuspended in PBS at a 100-fold concentration. Three different antigen preparations were done from this concentrate. (1) A crude ultrasonicated antigen containing viable organisms.

(Heating of the antigen at 56°C for 30 min which killed all *M. pneumoniae* organisms did not change the ability of the antigen to induce lymphocyte stimulation.) (2) A so-called membrane fraction obtained by repeated freezing and thawing and ultracentrifugation as described by Williams & Taylor-Robinson (1967). This preparation lacked viable organisms but it was not ascertained that it contained only membranes. (3) A crude lipid fraction obtained by chloroform–methanol extraction followed by KCl partition, evaporation and reconstitution with PBS (Kenny & Grayston, 1965). The antigen preparations were divided in small aliquots and stored at −70°C. The CF activity of these antigens was compared by checker board titrations with a human *M. pneumoniae* immune serum (Fig. 1). The CF antigen titres of sonicated antigen, membrane fraction and lipid fraction were 128, 8 and 32–64 respectively.

Lymphocyte stimulation

Human peripheral blood was collected, defibrinated by shaking with glass beads, mixed with 3% gelatine and allowed to stand at 37°C for 60 min. The leucocyte-rich supernatant was aspirated, washed three times, transferred to a column of nylon fibres (Leuco-Pak, Fenwal laboratories) and kept at 37°C for 30 min. The column was then eluted with pre-warmed medium RPMI 1640 (Biocult Laboratories, Paisley, Scotland). Erythrocytes in the eluate were lysed at 37°C for 20 min in a solution consisting of one part PBS and eight parts 0·8% ammonium chloride. The cells were counted. The cell population obtained usually consisted of 95–99% lymphocytes.

Cultures with and without various antigen preparations were set up in tubes containing 2×10^6 viable lymphocytes in 2 ml RPMI medium supplemented with 10% human AB serum (heated at 56°C for 30 min), penicillin and streptomycin. AB-serum from three donors, lacking CF and TRI antibodies to *M. pneumoniae* were used. It was checked that the lymphocyte response obtained by culture in medium supplemented with one or the other of these sera did not differ appreciably.

To sets of four to five tubes was added one of the following substances: (a) ultrasonicated *M. pneumoniae* antigen (final dilution 1/400 where not otherwise stated); (b) membrane antigen (final dilution 1/100); (c) lipid antigen (final dilution 1/25–1/200); (d) complete mycoplasma broth medium without mycoplasma (final dilution 1/500 or 1/1000); (e) phyto-haemagglutinin (PHA Difco 40 μg/ml). One set of tubes received no additives. The concentration of *M. pneumoniae* antigens chosen for the tests was the dilution giving an optimal lymphocyte response.

After 4 days of incubation at 37°C in 3% CO_2 and a relative humidity of 80–90% [^{14}C]thymidine (0·5 μCi/tube) (Amersham) was added to three (or two) tubes of each set of cultures. The cells were then harvested after overnight incubation. After two washes with cold PBS the cells were precipitated with 5% TCA and dissolved in 0·1 N NaOH. Instagel (Pachard) was added and the radioactivity was measured in a liquid scintillation counter (Philips) and expressed in counts per min (cpm). The mean value for triplicate (or duplicate) tubes was calculated. In each experiment the values obtained with lymphocytes cultured in the presence of added broth or in tissue culture medium alone was subtracted from the values obtained with lymphocytes cultured in the presence of *M. pneumoniae* antigen. The counts for lymphocytes cultured with the addition of broth was usually about the same as the counts for lymphocytes cultured without additives. Subtraction was done with the highest of these two values.

Lymphocyte stimulation was also assessed by counting morphologically transformed cells. Cells which had been cultured for 5 days were centrifuged directly onto glass slides in a cyto-centrifuge and stained with May–Grünewald–Giemsa. 500–1000 cells from each tissue culture tube were examined for transformation. The results were expressed as percentage of transformed lymphocytes. The percentage of transformed lymphocytes in cultures with no additive or with added broth was subtracted from the percentage of transformed lymphocytes in cultures with *M. pneumoniae* antigen.

Antibody tests

Sera were examined for antibodies to *M. pneumoniae* by CF using a *M. pneumoniae* lipid antigen (Kenny & Grayston, 1965) and by TRI (Senterfit & Jensen, 1966) as described previously (Biberfeld, 1971).

TABLE 1. *M. pneumoniae*-induced lymphocyte stimulation *in vitro* measured by [^{14}C]thymidine uptake and by morphological transformation in nine representative cases of recent *M. pneumoniae* infection

Case number	[^{14}C]Thymidine uptake (cpm) a Control cultures Mean	SE*	b Cultures with *M. pneumoniae* antigen Mean	SE	b−a	Ratio b/a	Percentage of transformed cells with *M. pneumoniae* antigen
1318	1539	62	18450	1725	16911	12·0	5·4
1494	3520	543	21513	3708	17993	6·1	12·5
1535	314	7	21971	891	21657	70·0	15·9
1247	1158	146	29966	942	28808	25·9	23·3
1152	716	1	32564	2934	31848	45·5	25·3
1498	2178	968	32872	57	30694	15·1	24·8
1270	2904	351	37026	4079	34122	12·8	22·4
182	479	40	41790	1967	41311	87·2	19·8
1585	2373	569	43428	1642	41055	18·3	28·0

* SE = standard error.

RESULTS

Quantitation of lymphocyte stimulation

The lymphocyte stimulation response is often expressed as the ratio between the isotope uptake in cultures exposed to antigen to the uptake in cultures without antigen. However, in our experimental system where the uptake in antigen-stimulated cultures was large the use of ratios for quantitation would have given misleading results as shown by the data presented in Tables 1 and 2. Stimulated cultures with approximately the same thymidine uptake had very different ratios because of the variation in the uptake in the unstimulated cultures from different donors. The degree of morphological lymphocyte transformation was better correlated to the cpm value than to the ratio.

Lymphocyte response in cases of M. pneumoniae infection and in healthy subjects

The *in vitro* lymphocyte response to three types of antigen preparations of *M. pneumoniae* and to PHA, as measured by [^{14}C]thymidine incorporation, in seventeen patients with recent *M. pneumoniae* infection examined 26–87 days after onset of illness and in ten serologically negative control subjects is summarized in Table 3. In some of these cases the lymphocyte reactivity to all the antigens was not examined. The lymphocyte response to sonicated *M. pneumoniae* antigen and to membrane antigen was significantly higher in recently infected patients than in healthy controls ($P<0{\cdot}001$ for sonicated antigen; $P<0{\cdot}01$ for membrane antigen). With sonicated antigen the highest value in the control group was 12,728 cpm. Sixteen out of seventeen patients had values above this level. However, nine out of ten control subjects showed some degree of lymphocyte response to *M. pneumoniae* antigen (Table 2).

TABLE 2. Lymphocyte stimulation *in vitro* in the presence of *M. pneumoniae* antigen measured by [^{14}C]thymidine incorporation and by morphological transformation in ten healthy subjects lacking antibodies to *M. pneumoniae*

Subject number	[^{14}C]Thymidine uptake (cpm) a Control cultures		b Cultures with *M. pneumoniae* antigen		b−a	Ratio b/a	Percentage of transformed cells with *M. pneumoniae* antigen
	Mean	SE*	Mean	SE			
158	2024	483	2644	376	620	1·3	1·1
17	684	143	2318	132	1634	3·4	0·7
36	918	83	3075	220	2157	3·3	0·6
114	357	83	2526	270	2169	7·0	2·0
109	252	42	5565	726	5313	22·1	8·0
9	522	88	6454	458	5932	10·1	4·0
84	2366	666	8150	122	5784	3·4	0·6
220	1203	79	8437	135	7234	7·0	5·1
37	655	269	12014	1290	11359	18·3	6·1
22	1461	29	14189	3685	12728	10·1	6·2

* SE = standard error.

The lymphocyte reactivity to membrane antigen corresponded closely to the response obtained with sonicated antigen (Table 3).

In contrast to the sonicated and the membrane antigens the lipid fraction of *M. pneumoniae* induced no appreciable stimulation of lymphocytes from infected cases or controls (Table 3). It is apparent that the lymphocyte-stimulating activity of *M. pneumoniae* antigens is not related to the CF antigen activity since the lipid fraction had a higher CF antigen titre than the membrane fraction (Fig. 1).

There was no significant difference in the lymphocyte response to PHA between patients and healthy controls (Table 3).

The lymphocyte responses to the various *M. pneumoniae* antigens measured by counting

TABLE 3. Lymphocyte stimulation, measured by [^{14}C]thymidine uptake (counts per minute), in response to various antigen preparations of *M. pneumoniae* and to PHA in cases with recent or previous *M. pneumoniae* infection and in control subjects lacking *M. pneumoniae* antibodies

	Sonicated antigen	Membrane antigen	Lipid antigen	PHA
Healthy subjects				
Number of subjects	10	6	5	10
Range	620–12728	1658–17916	34–240	10928–70155
Mean	5439	7692	124	36001
SD*	4095	7298	92	19644
Cases with recent infection				
Number of subjects	17	11	9	17
Range	12365–68621	9521–49362	1–1306	14687–76867
Mean	31002	28381	235	39713
SD	14870	13896	429	18110
Cases tested 5–6 years after infection				
Number of subjects	7			
Range	5139–49086			
Mean	24421			
SD	17312			
Cases tested 10 years after infection				
Number of subjects	4			
Range	7650–26475			
Mean	15984			
SD	7786			

* SD = standard deviation.

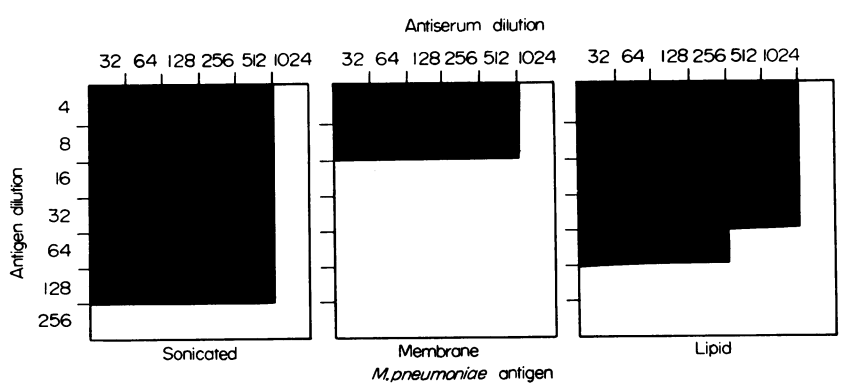

FIG. 1. Checker board titrations of three *M. pneumoniae* antigen preparations with a human immune serum to *M. pneumoniae*. The shaded area denotes the antigen and antiserum dilutions which gave complete fixation of complement. The dilutions are expressed as reciprocal values. (From Biberfeld (1972), with the permission of Associated Scientific Publishers, Amsterdam.)

of morphologically transformed cells (Table 4) were in general agreement with the results obtained by measurements of [^{14}C]thymidine uptake (Table 3). Quantitation of morphological lymphocyte transformation with sonicated antigen was performed in thirteen patients with recent *M. pneumoniae* infection and in ten control subjects. In the control subjects the percentage of transformed lymphocytes ranged from 0·6 to 8·0. In eleven out of thirteen cases of *M. pneumoniae* infection the degree of lymphocyte transformation ranged from 12 to 35%.

TABLE 4. Morphological lymphocyte transformation (percentage of transformed cells) in response to sonicated and lipid *M. pneumoniae* antigen and to PHA in cases with recent *M. pneumoniae* infection and in healthy subjects lacking *M. pneumoniae* antibodies

	Sonicated antigen	Lipid antigen	PHA
Healthy subjects			
Number of subjects	10	4	8
Range	0·6–8·0	0·1–0·5	37–89
Mean	3·4	0·3	61·1
SD	2·8	0·2	19·6
Patients with recent infection			
Number of subjects	13	5	8
Range	4·0–34·8	0–0·5	46–85
Mean	20·9	0·2	67·5
SD	9·1	0·2	15·4

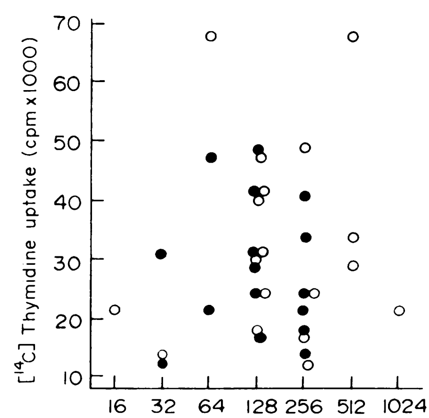

FIG. 2. *In vitro* lymphocyte response to *M. pneumoniae* antigen in relation to TRI (●) and CF (○) antibody titres in seventeen cases with recent *M. pneumoniae* infection.

The proportion of morphologically transformed cells was much higher in PHA-stimulated cultures than in antigen-stimulated cultures (Table 4), but the [^{14}C]thymidine uptake was almost as high in cultures stimulated by specific antigen as in cultures stimulated by PHA

(Table 3). This discrepancy is probably due to the fact that the thymidine uptake was measured on the 5th day of incubation, which is an optimal time for antigen-stimulated cells, while the peak thymidine uptake of PHA cells usually is found earlier.

Among the patients with *M. pneumoniae* infection there was no correlation between the magnitude of the *in vitro* lymphocyte response to *M. pneumoniae* antigen and the titre of TRI and CF antibodies (Fig. 2) (correlation coefficients 0·27 and 0·04 respectively).

Persistence of lymphocyte responsiveness and response to various doses of antigen

Four cases examined for lymphocyte reactivity 1–2 months after onset of *M. pneumoniae* illness were re-examined 1 year after infection. In three of these cases the lymphocyte response was approximately the same as during the early period after illness whereas in the fourth patient the response had decreased markedly.

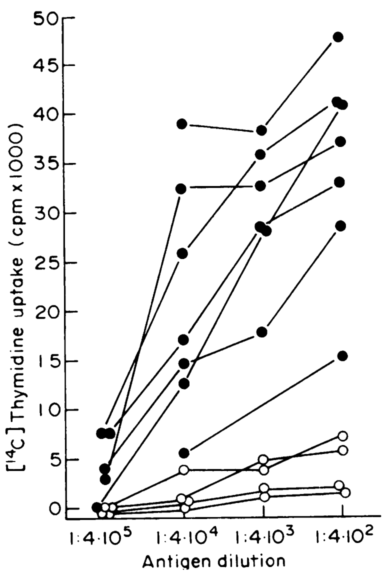

FIG. 3. Dose–response curves of lymphocytes from (○) four control subjects and (●) seven cases of *M. pneumoniae* infection cultured in the presence of sonicated *M. pneumoniae* antigen. The lymphocytes were collected 1–12 months after onset of illness.

The lymphocyte reactivity to various doses of sonicated *M. pneumoniae* antigen was examined in a series of patients and control subjects. The results are shown in Fig. 3. The thymidine uptake decreased with decreasing antigen concentrations but the dose–response curve showed a plateau in some of the recently infected patients. At the lowest antigen concentration used lymphocytes from the four control subjects tested showed no reactivity, while lymphocytes from four out of five cases of *M. pneumoniae* infection reacted.

The lymphocyte response to various doses of antigen was also measured 5–6 years after *M. pneumoniae* infection in nine cases (Fig. 4). These cases had been examined for *M. pneumoniae* antibodies but not for lymphocyte stimulation in the early period after infection. In this group of cases (two cases with reinfection excluded, see below) the lymphocyte response to the highest antigen concentration used (dilution 1/400) did not differ significantly from the response in cases examined 1–12 months after onset of *M. pneumoniae* illness (Table 3, Figs 3 and 4). However, at the intermediate antigen concentrations (dilution 1/4000 and 1/40000) the lymphocyte reactivity was somewhat lower in cases examined after 5–6 years than in cases tested early after infection ($P<0{\cdot}05$) (Figs 3 and 4).

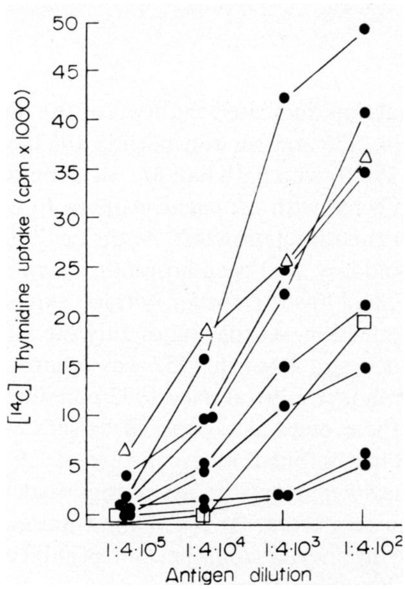

FIG. 4. (●) Dose–response curves of lymphocytes collected from nine cases 5–6 years after *M. pneumoniae* infection and cultured in the presence of sonicated *M. pneumoniae* antigen. Two cases ((□) father and (△) son, see text) had experienced a reinfection with *M. pneumoniae* 6 months before the lymphocyte test was performed.

TABLE 5. Stimulation of lymphocytes *in vitro* by sonicated *M. pneumoniae* antigen and TRI and CF antibody titres to *M. pneumoniae* in four cases examined 10 years after *M. pneumoniae* infection

Case number	Lymphocyte stimulation [¹⁴C]thymidine uptake (cpm)		Antibody titre	
	Mean	SE*	TRI	CF
278	26475	603	16	<4
277	14961	2223	<2	<4
270	14850	2710	64	<4
273	7650	807	<2	<4

* SE = standard error.

The lymphocyte reactivity to *M. pneumoniae* antigen was examined in four cases 10 years after they had had pneumonia due to *M. pneumoniae* (Table 5). All cases lacked CF antibodies to *M. pneumoniae* but two cases had TRI antibodies at a titre of 16 and 64 respectively. Three out of the four cases showed a higher degree of lymphocyte stimulation than control subjects. It should be noted that the lymphocyte reactivity was approximately the same in the case with a TRI antibody titre of 64 and in one of the cases which lacked *M. pneumoniae* antibodies. The mean lymphocyte reactivity to *M. pneumoniae* antigen (dilution 1/400) was lower in cases examined 10 years after infection than in recently infected patients ($P<0{\cdot}02$) (Table 3).

Reinfection

Anamnestic and serological data indicated that two of the nine cases examined 5–6 years after infection, a man born in 1933 and his son born in 1957 (included in a previous family study (Biberfeld & Sterner, 1969)) who both had *M. pneumoniae* pneumonia in September–October 1967, had been reinfected with *M. pneumoniae* in July 1972, 6 months prior to the collection of lymphocytes for the stimulation test. At the beginning of July 1972 the youngest child in the family, a 4-year-old boy, had been hospitalized with pneumonia and at the same time another son, born 1955, had lower respiratory tract illness associated with serological evidence of *M. pneumoniae* infection. At the end of July the father had a respiratory illness with severe cough, whereas the son born in 1957 was healthy. The serum specimens collected together with the lymphocytes in January 1973 contained a four-fold higher titre of TRI antibodies in both of these cases as compared to sera collected 3 years earlier. The father, but not the son, also had a four-fold rise in titre of CF antibodies. The lymphocyte response to sonicated *M. pneumoniae* antigen was rather weak in the father (Fig. 4) in spite of the fact that he had been reinfected. At the highest antigen concentration the isotope uptake was higher than for lymphocytes from the control subjects but with the lower antigen concentration there was no lymphocyte response.

DISCUSSION

The stimulation of sensitized lymphocytes when exposed to the specific antigen *in vitro* has been shown to correlate with cell-mediated immunity *in vivo* (Oppenheim, 1968) but lymphocytes involved in humoral immune responses also seem to take part in the stimulation response (Benezra, Gery & Davies, 1969).

In the present study it was shown that lymphocytes from patients with recent or previous *M. pneumoniae* infection became stimulated when cultured in the presence of a sonicated concentrate of *M. pneumoniae* organisms or a membrane fraction of *M. pneumoniae* but did not respond when exposed to a lipid fraction. The lipid fraction of *M. pneumoniae* contains the main CF activity (Kenny & Grayston, 1965) the active CF components being glycolipids (Plackett *et al.*, 1969) with haptenic properties (Sobeslavsky *et al.*, 1966). The inability of this haptenic fraction to stimulate the lymphocytes is in accordance with other observations that haptens without the carrier do not provoke transformation of immune lymphocytes (Mills, 1966; Oppenheim *et al.*, 1967).

Lymphocyte stimulation in response to whole *M. pneumoniae* organisms has previously been demonstrated in volunteers experimentally infected with *M. pneumoniae* (Leventhal *et al.*, 1968). In that study the observation period following infection was only about 1 month. Recently Fernald (1972) demonstrated stimulation of lymphocytes in cases of natural *M. pneumoniae* infection using killed, whole *M. pneumoniae* organisms as antigen in the test. Lymphocyte responsiveness was shown to persist for several years after infection, which was also found in the present study where some cases were tested as late after infection as 10 years. In the present study the lymphocyte reactivity to various doses of *M. pneumoniae* antigen was examined showing differences in the dose–response curves obtained with lymphocytes from recently infected patients as compared to the curves obtained with lymphocytes from cases tested several years after infection.

In contrast to Fernald, we observed *in vitro* lymphocyte reactivity to *M. pneumoniae* antigen also in the serologically negative healthy control subjects although the response was significantly lower than in the cases with serological evidence of recent or previous *M. pneumoniae* infection. However, lymphocytes from the sero-negative subjects responded only to high antigen concentrations. Thus, the discrepancy with the work by Fernald might depend on differences in the antigen concentrations used by Fernald and by us.

The present finding of lymphocyte responsiveness to *M. pneumoniae* in nine out of ten sero-negative cases suggests that these subjects had been infected with *M. pneumoniae* some time in the past. The ten sero-negative control subjects had been chosen for the lymphocyte test out of a group of 214 healthy blood donors, aged 18–50 years, where 83% of the donors had been found to possess TRI and/or CF antibodies to *M. pneumoniae*. Apparently there are very few people in this age group without some immunological evidence of previous *M. pneumoniae* infection.

Another interpretation of the lymphocyte reactivity to *M. pneumoniae* in serologically negative individuals cannot at present be excluded; thus it is possible that *M. pneumoniae* antigen in high concentrations has a nonspecific mitogenic effect, similar to that observed for some bacterial products, for example streptolysin S (Hirschhorn *et al.*, 1964).

Among the nine cases examined for lymphocyte reactivity 5–6 years after they had suffered pneumonia due to *M. pneumoniae*, two cases showed serological evidence of reinfection, one case having respiratory symptoms in association with the reinfection and the other case being asymptomatic. Data presented by other workers also indicate that reinfection with *M. pneumoniae* is not uncommon. In a study of marine recruits where the *M. pneumoniae* infection rate was 42%, 25% of the recruits with pre-existing TRI antibody were reinfected (Steinberg *et al.*, 1969). However, symptoms were usually mild in cases with reinfection. Foy *et al.* (1971) (Foy, personal communication) have reported two patients who twice suffered pneumonia due to *M. pneumoniae* with an interval of 4½ and 2 years respectively. There are thus observations from several studies suggesting that protective immunity to *M. pneumoniae* may not be long-lasting.

It is not known if humoral or cellular immune factors are most important for resistance to *M. pneumoniae* illness in man. Foy *et al.* (1973) recently reported that patients with antibody deficiency syndromes appear to have a more severe course of *M. pneumoniae* illness than immunologically competent persons. However, the fact that the antibody-deficient cases recovered and stopped excreting the organism suggested that part of the resistance to *M. pneumoniae* infection may be due to cell-mediated immunity (Foy *et al.*, 1973).

The lymphocyte stimulation test appears to be a sensitive method for the demonstration of cell-mediated immunity to *M. pneumoniae*. Further studies are needed to determine if the degree of *M. pneumoniae*-induced lymphocyte stimulation is directly related to the degree of resistance to infection. Lymphocyte responsiveness to *M. pneumoniae* persisted several years after infection but the response to suboptimal antigen concentrations was usually decreased. It is possible that the degree of lymphocyte responsiveness to various antigen doses may better reflect immunity than the magnitude of the response to a single high antigen dose.

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