of New York City was assisted by Miss Pat Ryan, Mrs. Dina Santana Bracco, Miss Sybil Gordon and Mrs. Margit Bloecker. Parke, Davis and Co. kindly provided 'Sernylan' used to immobilize the animals for examination.

The work was entirely supported by U.S. Public Health Service grants *GM*–12074–01, *FR*–00165 and *GM*–09237–03.

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# MYCOPLASMA (PLEUROPNEUMONIA-LIKE ORGANISMS) AND BLOOD GROUP I; ASSOCIATIONS WITH NEOPLASTIC DISEASE

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WE have reported that an association exists between the red blood cell group I and neoplastic disease, particularly leukaemia[1]. Whereas normal donors are rarely I negative (< 0·1 per cent), we found 38 of 124 patients with leukaemia to be I negative. Fluctuations in antigen I reactivity during exacerbation and remission in these patients suggested to us that the I antigen was blocked or destroyed during the course of the disease. There is one known relationship between an infectious disease and the I blood group, that is, the cold agglutinins of primary atypical pneumonia often have I specificity[2]. This prompted us to explore the disease relationship we observed in terms of possible association with micro-organisms.

In the work recorded here, 45 microbial agents including mycoplasma (PPLO or pleuropneumonia-like organisms), viruses and bacteria were added *in vitro* to I-positive red blood cells from normal donors to determine whether these agents could alter the I agglutinability of these cells. Eighteen of 25 mycoplasma tested, including three mycoplasma derived from human tumour tissue could block or destroy the I receptors of normal red cells *in vitro* and mimic our findings on patients with leukaemia.

The anti-I reagent was a γ-macroglobulin fraction of an isoimmune serum from an I-negative patient[1]. Parallel control tests were done with a human anti-$A_1$ (absorbed B) reagent.

The mycoplasma and bacteria were grown in broth medium containing a brain heart infusion base (Baltimore Biologics Laboratory), 15 per cent non-inactivated horse serum and 1 per cent yeast extract, incubated aerobically at 37° C for 3–5 days. The mycoplasma were sedimented by centrifugation at 27,000*g*, re-suspended in physiological saline at a 100-fold concentration and disrupted by freezing and thawing twice before use. The bacteria were sedimented at 8,000*g* and then treated in the same manner. The viral preparations were tissue culture suspensions except for the influenza viruses, which were chick embryo suspensions. They were also exposed to freezing and thawing. These microbial preparations had population titres ranging from $10^7$ to $10^9$ organisms per ml.

In the test system, one volume of the microbial material was added to an equal volume of a 2 per cent saline suspension of washed day-old I-positive human group $A_1$ red cells. The mixture was incubated at 22° C for 30 min and then washed three times in a large quantity of saline and reconstituted to a 2 per cent suspension. One drop of treated cell suspension was mixed with one drop of agglutinating reagent. The tubes were incubated at 22° C for 30 min and centrifuged. Macroscopic agglutination (+ + +) occurred with saline controls and was used as evidence for no inhibition. Lesser agglutination requiring microscopic examination indicated inhibition and was scored as trace inhibition (+ + agglutination) or strong inhibition (+ or negative agglutination). All tests were performed in parallel with cells and reagents from the same lots. Control tests were done using human group AB (non-immune) serum to detect any haemagglutination due to the microbial agents themselves. Broth medium and its yeast component were also tested for activity. After the preliminary experiments, the microbial materials and controls were number-coded before test and duplicate samples were intentionally included.

A total of 45 strains were tested as 58 unknowns for activity in both the I and A blood group systems. These included 25 mycoplasma strains (9 human, 5 murine, 2 avian and 1 each canine, calf and goat, 3 tissue culture and 3 saprophytes), 11 viral agents and 9 bacteria. Seven of the bacteria were non-specifically active, that is, caused pan-agglutination as in the Hubener–Thompsen phenomenon[3] when the group AB serum was added. These latter are not listed therefore in Table 1, which shows results of specific activity in the I blood group system of tested material. None of the other agents caused spontaneous haemagglutination with the exception of one influenza strain.

Anti-I agglutination was inhibited by 18 of the 25 mycoplasma strains tested (72 per cent); 7 of the 9 human strains were active (Table 1). One of the 11 viral agents had trace activity. *Streptococcus MG*, an organism used in serological tests for primary atypical pneumonia, was inactive. No inhibition of anti-$A_1$ was seen. (In other investigations, agglutination by anti-Lewis sera, $Le^b$ and $Le^a$, was not inhibited.)

The I agglutinability of red cells was altered by treatment with mycoplasma material for as little as 30 min. Stronger inhibition resulted when the treated washed cells were held overnight at 5° C. This may explain the vari-

ability of results seen with some of the mycoplasma, especially *M. hominis*, 2, and strain *Rab*-32.

It was not possible to demonstrate inhibition when mycoplasma were mixed with anti-I before addition of the indicator red cells. This indicates that the effect was not due to serologic cross-reactivity between PPLO and anti-I but was an alteration of the I receptor sites on the red cell.

Cold haemagglutinins are well known in patients with malignant disease of the lympho-reticular system and primary atypical pneumonia. These agglutinins often have anti-I specificity[2]. In primary atypical pneumonia it is presumed that *Mycoplasma pneumoniae*, the causative agent[4], is responsible for the development of the cold agglutinins. *Mycoplasma pneumoniae* and other PPLO produce haemolysis in culture[5]; others produce haem-adsorption[6].

Mycoplasma have been isolated from human tumour tissue. Horoszewicz recovered mycoplasma strain 880 from the spleen of a patient with chronic lymphocytic leukaemia[7], and one of us (M. F. B.) isolated mycoplasma strain *A*63–17 from lung tumour tissue of a patient with Hodgkin's disease and mycoplasma strain *RI*-12 from tissues of a patient with histiocytoma. These three strains of mycoplasma were among the agents showing the strongest inhibitory activity in the anti-I agglutination system.

Virus-like agents have been demonstrated in cell cultures and virus-like particles seen on electron microscopy of blood and bone marrow from patients with leukaemia[8–12]. Known mycoplasma under electron microscopy using the negative staining technique can appear quite similar to these particles[13]. Dmochowski reports that the blood of leukaemic patients which has virus-like particles also contains mycoplasma[14]. Murphy reported that his agent(s) which produces a fatal leukaemoid disease in mice is either a mycoplasma, an unidentified virus or both[15].

Table 1. Microbial Inhibition of the Agglutination by Anti-I

| Source | Identification | Inhibition of anti-I: None | Trace | Strong |
|---|---|---|---|---|
| *Mycoplasma* | | | | |
| Human sarcoma | Strain *A*63–17 | | | XXX |
| Human leukaemia | Strain 880 | | | XX |
| Human histiocytoma | Strain *RI*-12 | | X | XX |
| Human pneumonia | *M. pneumoniae* | | | XX |
| Human synovitis | Strain 6*R* | | X | X |
| Human penis | *M. fermentans* | | X | X |
| Human mouth | *M. salivarium* | X | | |
| Human urethra | *M. hominis*, 1 | X | | |
| Human urethra | *H. hominis*, 2 | X | | X |
| Murine arthritis | *M. arthritidis* | | | |
| Murine arthritis | Strain *JR*-3 | | X | |
| Murine arthritis | Strain *Preston* | X | | |
| Murine lung | *M. pulmonis* | X | | |
| Murine brain | *M. neurolyticum* | | | |
| Murine brain | Strain *PG*-28 | X | | |
| Murine brain | Strain *A* | | X | |
| Avian lung | *M. gallinarum* | X | | |
| Avian sinusitis | Strain *NTF* | | X | X |
| Canine | *M. spumans* | | | X |
| Bovine | Strain *'calf'* | | X | |
| Goat | Strain *'kid'* | | | X |
| Tissue cell culture | Strain *RK*-13 | X | | |
| Tissue cell culture | Strain *Hep*-2 | | | XX |
| Tissue cell culture | *Rab*-32 | X | | X |
| Saprophyte | *M. laidlawii, A* | X | X | |
| Saprophyte | *M. laidlawii B* | | XX | |
| Saprophyte | Strain *Laidlaw* | | | X |
| *Virus* | | | | |
| Poliovirus | Type 1, *TA* 2 | X | | |
| Poliovirus | Type 2, *TB* 2 | | X | |
| Poliovirus | Type 3, *TC* 2 | X | | |
| Adenovirus | Type 3, *GB* | X | | |
| Adenovirus | Type 4, *MA* | X | | |
| Adenovirus | Type 7, *LL* | X | | |
| Influenza | A/swine/1976/31 | X | | |
| Influenza | A2/Japan/305/57 | X | | |
| Influenza | A2/Japan/170/62 | X | | |
| Influenza | B/Maryland/1/59 | X | | |
| Smallpox | Reference Lot 2 | X | | |
| *Bacteria* | | | | |
| | *A. aerogenes* | X | | |
| | Streptococcus MG | X | | |
| *Control* | | | | |
| | Broth medium | X | | |
| | Yeast extr. 25% | X | | |

X = individual test result on separate specimen.

Our findings suggest that an association may exist between the red cell antigen I, mycoplasma and leukaemia. Of course, it does not follow necessarily that mycoplasma are involved in the pathogenesis of this disease. Mycoplasma are important agents in veterinary medicine. They have been isolated from patients with non-gonococcal urethritis[16], Reiter's syndrome, rheumatoid arthritis and lupus erythematosus[17]. However, primary atypical pneumonia remains the only disease of man known to be caused by mycoplasma. Thomas has suggested that since lung tissue antibody appears in patients with primary atypical pneumonia, the possibility that infection with mycoplasma is implicated in conditions characterized by auto-immune serologic reactions requires further investigation[18]. Our finding that red cell antigen I can be altered *in vitro* by mycoplasma when considered with the findings of both mycoplasma[4] and anti-I antibodies[2] in primary atypical pneumonia is an illustration of this concept.

The *in vitro* interference with anti-I agglutination by mycoplasma presents a working hypothesis to explain the inagglutinability by anti-I of red cells of patients with leukaemia. We suggest that although mycoplasma may not be primary agents in human neoplastic disease, they might be opportunist or secondary invaders. They could be involved in combination with another (viral?) agent, as in the mouse hepatitis system[19] or the Rous-helper system[20].

One other microbial agent is known to alter the I red cell antigen. Marcus[21] found that filtrates of *Clostridium tertium* can destroy the I determinant. These filtrates were specific for I but were more active against the I factor of group A cells than of group O cells. The activity was probably enzymatic. We have found that mycoplasma can alter I antigen reactivity and that they are also more active against the I factor of group A cells than of group O cells. PPLO are enzymatically active, especially in the arginine dehydrolase system[22], and the *in-vitro* effect we have observed may be due to an enzymatic degradation of the I antigen determinant on normal cells.

We thank Dr. Yokoyama, who prepared the γ-macroglobulin fraction, Drs. J. Fabricant, J. A. Morris, J. Nelson, H. Neimark, J. Seligman and J. Tully, who supplied some of the microbial agents, and D. B. Riggs and M. W. Grabowski for their help.

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