Mycoplasma Inhibition of Phytohemagglutinin Stimulation of Lymphocytes

Abstract. *Goat lymphocytes were cultured in vitro with phytohemagglutinin and nonviable mycoplasmas. Addition of the mycoplasmas, even as late as 45 hours after adding phytohemagglutinin, completely inhibited the increase in synthesis of DNA and RNA normally induced in lymphocytes by the mitogen. The suppression of synthesis did not result from killing of the cells by the mycoplasmas, combination of the organisms with phytohemagglutinin, or competition for combining sites on the cell surface, which indicates that some other mechanism of inhibition was operative. A similar depression of response to phytohemagglutinin in lymphocytes in culture has been observed in human diseases associated with an immune defect. The present demonstration that at least certain mycoplasmas can profoundly affect lymphocyte function in vitro suggests that thay may alter the immune response in vivo.*

Lymphocytes cultured in vitro in the presence of phytohemagglutinin (PHA) respond by morphologic transformation into blast-like forms (*1*) and by increased synthesis of DNA and RNA (*2*), as measured by incorporation of radioactive labeled precursors into the acid-precipitable residue (*3*). Copperman and Morton demonstrated that mycoplasma reversibly inhibit the PHA-induced morphologic transformation of lymphocytes cultured in vitro (*4*). In the present study we investigated the effects of mycoplasma on DNA and RNA synthesis in lymphocytes in vitro.

Lymphocytes were obtained from lymph collected from an adult Angora goat by means of a thoracic duct–venous shunt (*5*). The thoracic duct lymph was continuously recirculated into the venous system, but could be tapped at will, yielding a cell population comprised of more than 95 percent small lymphocytes. The lymph was centrifuged at 1000 rev/min for 10 minutes. Lymphocytes were suspended in Eagle's minimal essential medium for suspended cultures (Spinner's medium) containing 100 units of penicillin and 100 μg of streptomycin per milliliter, 1 percent L-glutamine, and 15 percent calf serum. The suspension was adjusted to a final concentration of 2.5×10^5 lymphocytes per milliliter. All cultures were prepared in triplicate with 4 ml of cell suspension per tube. Phytohemagglutinin-M (Difco), 0.1 ml, was added at the initiation of culture. Selected cultures were inoculated as indicated with *Mycoplasma arthritidis* strain PN (5×10^7), which had been killed either by lysis in distilled water or by heating at 60°C for 1 hour.

For assays of DNA synthesis, C^{14}-thymidine, 0.1 μc, was added to the cultures after incubation for 48 hours. Twenty-four hours later, the cells were harvested in the cold by serial suspension in iced saline, 5 percent trichloroacetic acid (twice), and methanol. The acid-precipitable residue was dissolved in Hyamine (*6*), transferred to counting vials containing scinitillation fluid in toluene, and counted in a Packard Tri-Carb scintillation counter. The results obtained on triplicate cultures were averaged and expressed as counts per minute per culture.

Assays of RNA synthesis were performed similarly. Uridine labeled with C^{14}, 0.1 μc, was added after 5 hours of incubation, and the cells were harvested 24 hours later.

Addition of the mycoplasmas to the cultures completely inhibited PHA stimulation of DNA synthesis, as evidenced by near base-line incorporation of C^{14}-thymidine (Fig. 1). In some experiments the cells were washed on day 2 and resuspended in fresh medium without further addition of PHA. In these cultures, inhibition of DNA synthesis was reversed and cellular stimulation occurred. Similarly, RNA synthesis was inhibited when PHA and the mycoplasmas were added simultaneously to the cultures. When the mycoplasmas were removed by washing the cells after a 5-hour incubation period, stimulation again occurred with no further addition of PHA.

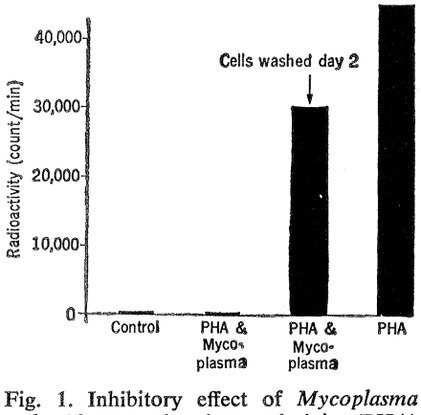

Fig. 1. Inhibitory effect of *Mycoplasma arthritidis* on phytohemagglutinin (PHA) stimulation of DNA synthesis in goat lymphocytes in vitro, as measured by incorporation of C^{14}-thymidine.

These data show that nonviable mycoplasmas inhibit both RNA and DNA synthesis in PHA-stimulated lymphocytes and that the inhibition can be reversed by washing the cells. In both assays, the response on removal of the mycoplasmas indicates that the cells were viable and metabolically active. Furthermore, since additional PHA was not required for cell response, the inhibitory effect was not mediated by inactivation of PHA by the mycoplasmas.

The possibility that the mycoplasmas were competing with PHA for combining sites on the cell surface was then evaluated in a time study. A series of cultures were set up in triplicate tubes. Phytohemagglutinin was added to each tube at the initiation of culture. The mycoplasmas were added simultaneously with the PHA to one set of cultures; the remaining cultures were inoculated with the microorganisms at 1, 2, 4, 6, 20, 25, 35, and 45 hours after the addition of PHA. Thymidine labeled with C^{14} was added at 48 hours, and the cells were harvested at 72 hours. As shown in Fig. 2, significant inhibition of PHA-stimulated DNA synthesis occurred with each addition of the mycoplasmas, even as late as 45 hours after the addition of PHA. Since these results ruled out competition between PHA and the mycoplasmas for combining sites on the cell, some other mechanism of inhibition, such as direct suppression of DNA and RNA synthesis, must be operative.

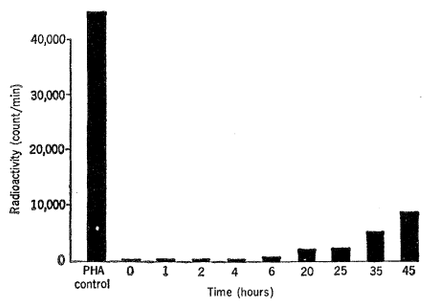

Fig. 2. Time course of inhibition by *Mycoplasma arthritidis* of phytohemagglutinin (PHA) stimulation of DNA synthesis in goat lymphocytes. Phytohemagglutinin was added to all tubes at the initiation of culture. Mycoplasmas were added simultaneously to one set of cultures; the remaining tubes were inoculated at the time indicated (in hours) after the addition of PHA.

Certain mycoplasmas, including *M. arthritidis*, are able to convert arginine to ornithine by way of the three-enzyme arginine dihydrolase pathway (*7*). Kraemer (*8*) showed that some strains of *Mycoplasma* that are cytocidal to lymphoma cells in culture liberate a toxic substance into the medium. This substance may act by depleting the medium of arginine, since the cytotoxic effect can be prevented by the addition of arginine to the cultures. Whether the toxin is identical to one of the enzymes in the arginine dihydrolase pathway is not known. In our studies only nonviable mycoplasmas were used, eliminating the possibility that a toxin was produced during the culture period. Whether an inhibitory factor was released into the culture medium during the growth of the organism was not investigated. The present studies do not exclude the possibility that a heat-stable toxin is involved in the inhibition of PHA response, although the observation that lymphocytes exposed to the mycoplasmas for 48 hours were still capable of responding to PHA indicates the inhibitory factor was not cytocidal. The possibility remains, however, that the inhibition is produced by a heat-stable enzyme, which may act by depleting the medium of arginine. We have studied only one species of *Mycoplasma*, and since *Mycoplasma* belongs to an extremely heterogeneous family of organisms, our results cannot be assumed to apply to mycoplasmas in general.

The responsiveness of lymphocytes in vitro appears to be correlated with the immunologic competence of the host. A depressed response to PHA in lymphocytes in culture has been noted in diseases associated with a defect in immune response, such as Hodgkin's disease (*9*), chronic lymphocytic leukemia (*10*), sarcoidosis (*11*), agammaglobulinemia (*12*), and ataxia telangiectasia (*13*). The action of *M. arthritidis* on the immune system is not known, but it is possible that this organism produces an impairment of immune response in vivo comparable to the impairment of lymphocyte response demonstrated in vitro. Such a correlation has been observed in viral diseases. Montgomery *et al.* (*14*) demonstrated that the lymphocytes of patients with congenital rubella do not respond normally to PHA and found that the addition of rubella or Newcastle virus to cultures of normal lymphocytes inhibits their response to the mitogen. Smithwick and Berkovich (*15*) showed that measles virus inhibits the in vitro response to tuberculin purified protein derivative in lymphocytes from children having a positive reaction to this tuberculin, and they were able to correlate this effect with decreased reactions to tuberculin skin tests in tuberculous children during measles infection. Our studies were concerned only with the inhibition of PHA response; the effect of *M. arthritidis* on antigenic stimulation of sensitized lymphocytes awaits further investigation.

Mycoplasma infection has been postulated in the pathogenesis of a number of diseases of unknown etiology, including systemic lupus erythematosus, Reiter's syndrome, and rheumatoid arthritis (*16*). Although our data provide no information on the possible pathogenetic role of mycoplasmas, they do demonstrate that at least one species of *Mycoplasma* can profoundly affect lymphocyte function in vitro. Whether lymphocyte function is similarly affected in mycoplasma infections in man remains to be established.

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17. We thank Dr. Lewis Thomas and Dr. Evangelia Kaklamanis for supplying the *Mycoplasma arthritidis* (strain PN) used in this study and Darlene Dudley for technical assistance. Supported by NIH training grant HE-05677 (to L.S.), by funds from the Research Evaluation and Allocation Committee (Gilbert Fund, to K.C., and Simon Fund) of the University of California Medical Center and the Northern California Chapter of the Arthritis and Rheumatism Foundation, and by contract Nonr-3656(12) with ONR.

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Inherited C′2 Deficiency in Man: Lack of Immunochemically Detectable C′2 Protein in Serums from Deficient Individuals

Abstract. *Monospecific antiserum to highly purified second component of human complement (C′2) was used to show the absence of the protein from the serums of four persons homozygous for a hereditary deficiency of second-component activity. Serum from an individual heterozygous for the deficiency contained a reduced amount of this protein as compared to the concentration in normal serum. These observations indicate that genetic deficiency of this component is due to failure of synthesis of normal amounts of the protein rather than to synthesis of an antigenically related, hemolytically inactive analog of C′2.*

A deficiency of the second component (C′2) of human complement in a clinically healthy individual (*1*) was later shown (*2, 3*) to be an inherited characteristic. Three families with this deficiency have been described, and in each family the propositus was a homozygote for the defect (*2–4*). Several independent assays have established the presence of a small amount of second-component activity (usually less than 5 percent of normal) in the serums of the homozygous individuals. The serums from the heterozygotes showed approximately 50 percent of the normal activity (*1–5*).

Klemperer *et al.* (*3*) postulated that this deficiency was due to absence of the active protein rather than to the presence of an inhibitor of second-component activity. In our study, comparative immunochemical analyses were performed on both C′2-deficient and normal serums to determine directly whether the deficiency of the component in these serums was due to lack of the C′2 protein or to the presence of an inactive analog. It was presupposed that the potent, monospecific antiserum to normal C′2 used would detect a C′2 variant by immunochemical cross-reaction.