climatic region than between flows in different climates.

The technique, applicable to porphyritic glass also, is inexpensive and rapid; one person can make and measure about six thin sections (0.051 to 1.016 mm in thickness) per hour.

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Immunosuppressant from Group A Streptococci

Abstract. *A cytoplasmic component of group A streptococci suppresses both 19S and 7S antibody responses of mice to sheep erythrocytes. Partial purification is achieved by differential centrifugation and gel filtration. When the direct and indirect hemolytic plaque techniques are used, a single injection of this group A material given before injection of erythrocytes produces more than 90-percent suppression of either primary or secondary immune response.*

A potent immunosuppressant has been derived from a microorganism which is very common in our environment and which is a natural pathogen for man. Unlike most other immunosuppressants, a single injection of this material, when given before antigen, can produce significant suppression of the immune response, in doses far smaller than the usual lethal dose.

The immune response of mice to sheep erythrocytes was studied by the direct and indirect hemolytic plaque techniques to measure cells that produce 19*S* and 7*S* (*1*) antibody in the primary and secondary responses (*2*, *3*). Injections of streptococcal fractions and sheep erythrocytes (5×10^8) were given intraperitoneally. Each experimental group consisted of eight to ten mice; an equal number of control animals injected with saline and with sheep erythrocytes was included with every experimental group. All mice, 10 to 12 weeks of age, were either of Swiss-Webster strain (Carworth Farms) or from a closed-colony strain bred for 20 years at the University of North Carolina. Although most unsensitized adult mice show a background of antibody-forming cells in the spleen and probably have had previous contact with antigens cross-reactive with sheep erythrocytes, the terms primary and secondary indicate immune response after first and subsequent injections of antigen, respectively. The two responses are clearly distinguishable with regard to relative numbers of cells producing 19*S* and 7*S* antibody, as well as in the time required for the maximum increase of these cell types. In addition, there has been evidence that background antibody-forming cells are not related to the antigen-sensitive precursor cells of the primary response (*4*). The active streptococcal fraction described below increased the number of background plaque-forming cells (PFC) about tenfold. This stimulation, rather than suppression, of background is similar to that reported for phytohemagglutinin (*4*).

Group A, type 3, strain D-58 streptococci were cultured in Todd-Hewitt broth (*5*), washed three times with saline, disrupted in the Braun shaker, and subjected to differential centrifugation (*6*). Samples of supernatant and sediment were obtained after serial centrifugation for 45 minutes at 12,000, 37,000, and 100,000*g*. Mucopeptide extracted from the cell-wall fraction (*7*) was also prepared. The sedimentable fractions were washed twice with buffer and three times with distilled water. All fractions were dialyzed against distilled water and freeze-dried. These fractions were compared for their immunosuppressive effect by intraperitoneal injection of 5 mg into mice (groups of ten) 24 hours before injection of sheep erythrocytes. Spleen cells were obtained from the mice 4 days after erythrocyte injection and suspended in Hanks's solution for hemolytic plaque counts.

Only the soluble material in the supernatant fractions from centrifugation at 37,000 or 100,000*g* gave more than 50-percent suppression of 19*S* PFC. The supernatant from centrifugation at 37,000*g* was fractionated by ascending gel filtration on Sepharose 2B (*5*). Material was eluted from the column with pyrogen-free 0.15*M* NaCl at a flow rate of 10 to 12 ml/hr. The second fraction, designated SF-II, constituted 30 percent of the starting material, and 400 μg or more produced nearly complete suppression of the 19*S* PFC (97 percent); 200 μg produced 71-percent suppression. These observations were made 4 days after erythrocyte injection. The suppressive effect of the SF-II fraction on the primary 19*S* response was also studied after an 8-day interval to determine whether the 19*S* response had been delayed rather than suppressed. At this interval, the 19*S* PFC count was suppressed 60 percent. Although the SF-II cannot be considered a homogeneous fraction, it does represent considerable purification in that an antiserum to the soluble component of group A streptococcus which showed six precipitin lines in immunodiffusion with the crude supernatant after centrifugation at 37,000*g*, gave no precipitin lines with SF-II.

Although 1 mg of SF-II produced complete suppression when it was given 1 day before the erythrocytes, partial

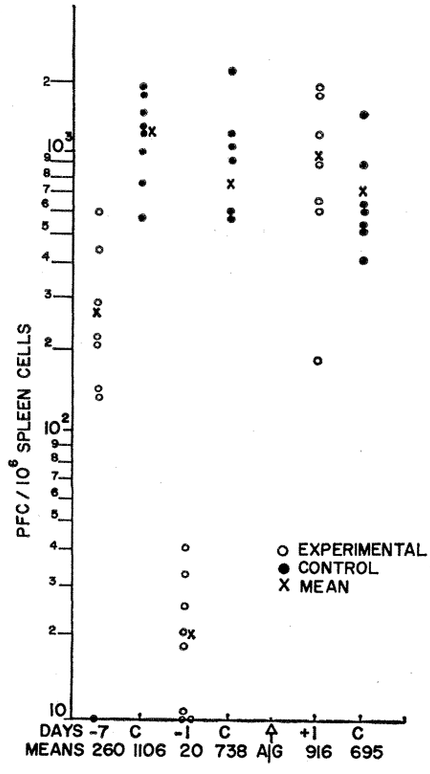

Fig. 1. Effect of interval between intraperitoneal injections of 1 mg of SF-II and of erythrocyte antigen on 19*S* PFC. Erythrocytes were injected at day 0 (A↑G), and spleen cell suspensions were prepared 4 days later. Each point represents one mouse.

suppression was still observed when SF-II was given 7 days before the antigen. There was no suppression when it was given 1 day after the antigen (Fig. 1).

We also examined the capacity of the streptococcal material to suppress 7*S* antibody-forming cells. When SF-II was injected 24 hours before the primary injection of erythrocytes, the 7*S* PFC were suppressed 90 percent (Fig. 2A). This was determined at an interval of 8 days after erythrocyte injection, which is the time of maximum 7*S* PFC response in immunized control mice. Approximately 90 percent suppression of 7*S* PFC was also obtained in the secondary response when SF-II was injected 1 day before the second injection of erythrocytes (Fig. 2B). Comparable degrees of suppression were observed with spleen cell suspensions prepared 2 and 4 days after the erythrocyte injection.

In spite of the fact that the primary 19*S* PFC response was nearly completely suppressed to background level, "memory" cells developed normally, as demonstrated by the following experiment. The SF-II was injected 1 day before the first sensitizing dose of sheep erythrocytes, and the second injection of erythrocytes was given 21 days later. The SF-II was given only before the first injection of erythrocytes. Both 19*S* and 7*S* PFC were determined 2 days after the second injection of erythrocyte antigen. As shown in Fig. 2C, a typical 7*S* secondary response was produced.

Most immunosuppressant agents act either through cytotoxic or antimetabolic properties (*8*, *9*) and frequently must be used in repeated large doses, especially to produce suppression of the secondary response (*9*). A feature of the immunosuppressant fraction from group A streptococci is its lack of toxicity. Even with a 5-mg dose, which is 25 times the amount that gives 70-percent suppression in a 25-g mouse, there was no grossly apparent toxic effect. With doses of 1 to 5 mg, there is an increase in total cell count and weight of the spleen. With regard to this apparent stimulation of cells and in several other features of its immunosuppressant effect, the streptococcal material resembles phytohemagglutinin. Major differences, however, include the more transient nature of phytohemagglutinin suppression and the necessity for its use in a near toxic dose (*10*).

We conclude (i) that the streptococcal fraction can suppress both primary and secondary responses if given before the first or second injection of antigen, respectively; (ii) that it cannot affect cells already stimulated by antigen because it has no influence on antibody production when given 1 or 2 days after antigen; and (iii) that it does not suppress the induction of immunological memory. With a system proposed by Nossal and Austin (*11*), we find that this material apparently acts on potentially competent stem cells (x) or "memory" cells (y) to prevent their differentiation to antibody-producing cells (z), although it does not interfere with differentiation from x to y. The mechanism by which this may be accomplished is still unknown.

We still have no information on the distribution of this activity among other bacteria. Hanna and Watson (*12*) described the immunosuppressant effect on rabbits to whom pyrogenic exotoxin from group A streptococci was given after erythrocytes. We have not determined the pyrogenicity in rabbits of the material reported here, nor do we have enough information regarding its physicochemical properties to determine the possible relation of our material to that reported by Hanna and Watson (*12*). It has been reported that endotoxin from gram-negative bacteria selectively damages small lymphocytes indirectly through adrenal hormones (*13*). Wang and Neter (*14*) also reported that endotoxin can selectively suppress production of antibody by rabbits against a bacterial antigen, if the endotoxin and antigen are injected together. We are not aware of any other reports relating products of pathogenic microorganisms to suppression of immune response. The unique significance of immunosuppressant activity associated with bacteria common in our environment is that it represents a natural but exogenous source of control of the immune response.

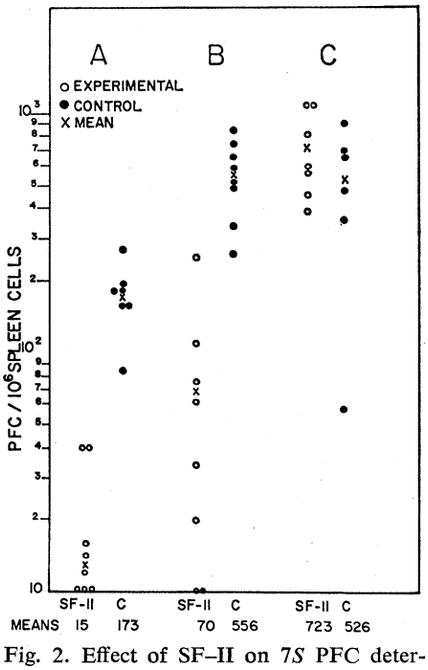

Fig. 2. Effect of SF–II on 7*S* PFC determined by indirect hemolytic plaque technique. The net number of 7*S* PFC was obtained by subtracting the number of 19*S* PFC from the total number of PFC obtained by the indirect technique. (A) Primary immune response. The SF-II (1 mg) was injected 24 hours before injection of erythrocytes, and the spleen cell suspension was prepared 8 days after erythrocyte injection. (B) Secondary response. The SF-II (1 mg) was injected 24 hours before the second injection of erythrocytes. No SF-II was given before primary injection. The interval between primary and secondary antigen was 21 days. The spleen cells were obtained 4 days after erythrocyte injection. (C) Anamnestic response. The SF-II (1 mg) was given 24 hours before primary injection of erythrocytes. The second injection of erythrocytes was given 21 days later. The spleen cells were obtained 2 days after second dose of erythrocytes.

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References and Notes

1. In conventional terminology, the terms 19*S*, or γM, refer to antibody-forming cells that are measured by their capacity to produce a hemolytic plaque by the direct technique (*2*). The term 7*S* PFC refers to those cells that produce hemolytic plaques when antibody to mouse globulin is added [the indirect technique (*3*)]. Our rabbit antibody to mouse globulin had no antibody to γM, and in a dilution of 1:150 it did not reduce the 19*S* plaque count. We use the term 7*S* because our antiserum does not distinguish between mouse immunoglobulins γF, γG, or γH.
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