Transformation of Human Lymphocytes by *Haemophilus influenzae* Somatic and Polysaccharide Antigens[1] (37414)

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Cell-mediated immune reactions directed against *Haemophilus influenzae* antigens include delayed-type skin test reactivity (1), and *in vitro* transformation of human lymphocytes. The latter has been reported employing human lymphocytes and an antigen prepared from a nonspecified type of *H. influenzae* (2). Significant *in vitro* human lymphocyte transformation utilizing a soluble complex somatic antigen prepared from a nontypable *H. influenzae* strain has been confirmed by this laboratory (3). The present study investigates the capacity of soluble antigens prepared from both nonencapsulated nontypable, and encapsulated type b organisms to induce transformation of human lymphocytes and compares their effectiveness to the transforming capacity of type b polysaccharide.

Materials and Methods. H. influenzae *strains.* Encapsulated type b (ATCC 9795) (kindly provided by Dr. Sarah H. Sell, Vanderbilt University Medical School, Nashville, TN) and a nontypable strain (typing performed by the laboratory of Dr. Sarah H. Sell) obtained from an adult with bronchiectasis were utilized.

Antigens. The complex soluble natural antigenic extracts described herein are designated somatic antigen. Their method of preparation has been previously described (3). Briefly, *H. influenzae* organisms from overnight cultures in Levinthal's broth were disrupted by 15–18,000 lb pressure in a French type laboratory press (American Instrument Co.). Extracts were clarified by centrifugation at 800*g* for 20 min, ultracentrifuged at 100,000*g* for 1 hr, dialyzed 3 times against 0.15 *M* NaCl, once against Hanks' buffered salt solution (HBSS) and filtered through a 0.45 μm bacteriological filter (Millipore). The resulting somatic antigens had dry weights of approximately 1.5 mg/ml, contained protein (62% antigen from nonencapsulated, 47% antigen from encapsulated strain) as determined by the Lowry *et al.* method (4), and included considerable nucleic acid as indicated by an ultraviolet absorption ratio at 280 nm to that at 260 nm of 0.84. The somatic antigens were virtually free of endotoxin-like activity as previously reported (3). Somatic antigen was stored at 4° and used within a 4 mo period. The polysaccharide antigen was polyribose phosphate vaccine (kindly provided by Dr. Porter Anderson, Boston Children's Hospital, Boston, MA) which had been aseptically dialyzed three times against 0.15 *M* NaCl and once against HBSS. The dry weight of this antigen was 200 μg/ml. It was stored at 4° and used shortly after dialysis.

Lymphocyte transformation. Venous blood was obtained from healthy adult volunteers and lymphocytes were prepared as previously reported (5). Lymphocytes from the effluent carefully eluted from cotton-filled polypropylene pipettes (0.025 ml pipette dropper, Cooke Eng. Co.) were approximately 95–99% pure accompanied by some other mononuclear cells and occasional granulocytes and were virtually 100% viable as determined by trypan blue exclusion. Cultures containing 10^6 lymphocytes in 2 ml media 199 (Flow Laboratories) supplemented with 20% autologous plasma were incubated 6 days at 37°

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TABLE I. Lymphocyte Transformation by Somatic Antigens of Encapsulated and Nonencapsulated *H. influenzae*.

Somatic antigen (μg/culture)	Tritiated thymidine uptake (cpm/cultures)	
	Type b	Nontypable
0	2725	2725
5	13,180 (4.8)[a]	11,740 (4.3)
20	32,080 (11.8)	33,080 (12.1)
80	56,610 (20.8)	49,215 (18.1)

[a] The number in parentheses is the stimulation index (*S/C*), the ratio of the antigen-stimulated (*S*) to unstimulated control (*C*) cultures.

in a humidified atmosphere of 5% CO_2. Two microcuries of tritiated thymidine (New England Nuclear, sp act 6.7 Ci/mole) were added during the last 24 hr of incubation. Termination of cultures and determination of the thymidine uptake by liquid scintillation counting of the trichloroacetic acid-insoluble residue as an indicator of DNA synthesis was by previously described methods (5).

Results. A comparison of lymphocyte transformation induced by somatic antigen prepared from a nonencapsulated strain to that of the encapsulated type b *H. influenzae* strain is indicated in Table I. Both strains yielded somatic antigens of equal lymphocyte stimulating potency causing significant transformation over a wide range of concentrations. No appreciable difference in transformation was observed between antigens from the two strains despite somewhat higher protein concentration in antigen from the nonencapsulated, nontypable strain. All subsequent results with somatic antigen reported herein were obtained using antigen prepared from the nontypable strain.

A dose–response curve comparing lymphocyte transformation due to somatic and polysaccharide antigens is depicted in Fig. 1. Somatic antigen caused remarkedly greater stimulation than did polysaccharide. As little as 1.25 μg/culture yielded significant blastogenesis which was optimal at approximately 40 μg. Higher concentrations of somatic antigen yielded inconsistent results. In some experiments higher concentrations resulted in suboptimal transformation and in others greater transformation than did 40 μg. In contrast the polysaccharide-induced transformation was of a significant degree only at the highest concentration tested—40 μg/culture. Lesser amounts of polyribose phosphate resulted in virtual absence of transformation under the conditions of these experiments.

The results from somatic antigen-induced transformation with lymphocytes obtained from 10 normal adults are depicted in Fig. 2. Somatic antigen (40 μg) was added to these cultures. Lymphocytes from all subjects tested responded *in vitro* with at least threefold increase in tritiated thymidine uptake indicating considerable blastogenesis elicited by the somatic antigen. The mean degree of stimulation was 12.6 times that of unstimulated control cultures.

Discussion. Considerable transformation occurs when lymphocytes of normal adults are exposed *in vitro* to somatic antigen of *H. influenzae* with virtual absence of reactivity in response to purified polyribose phosphate *H. influenzae* type b capsular polysaccharide.

Immunity to *H. influenzae* type b is understood to result primarily from serum anticapsular antibody. Formation of this antibody is due to either inapparent infection with type b organisms or infection with cross-reactive gram-positive and -negative bacteria (6). Anti-type b antibodies also may be raised in adults and infants by vaccination with purified *H. influenzae* type b capsular

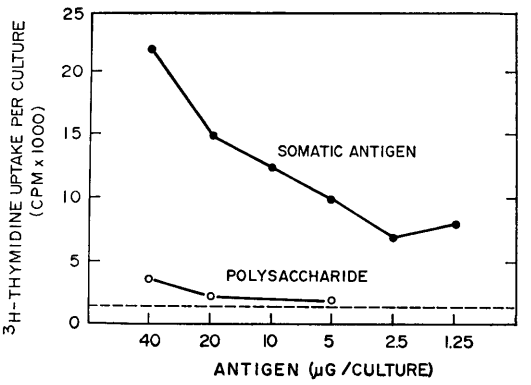

FIG. 1. Dose–response curve of lymphocytes from a representative normal adult. Responses to the somatic antigen and polysaccharide were determined concomitantly. (— —) The thymidine uptake of the donor's unstimulated lymphocytes.

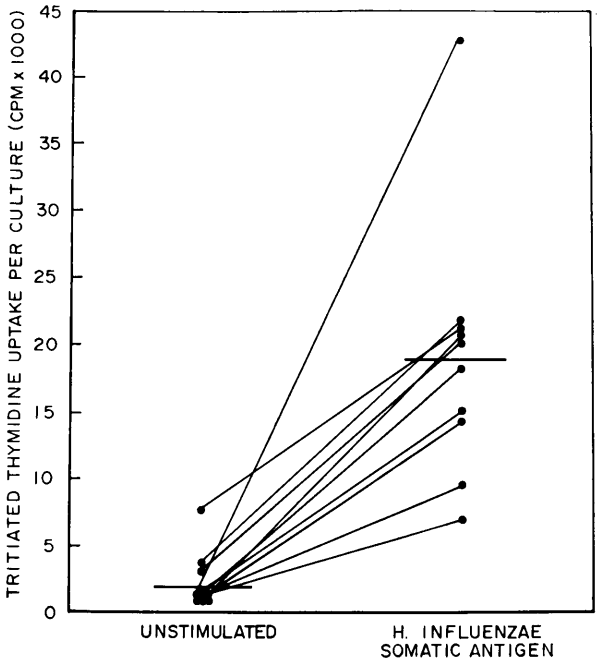

FIG. 2. Lymphocyte transformation of lymphocytes from 10 normal adult donors induced by somatic antigen prepared from a nontypable *H. influenzae* strain. Forty micrograms of antigen were added to stimulated cultures. Horizontal lines: the means of unstimulated and somatic antigen-stimulated cultures.

polysaccharide, polyribose phosphate (6). Antibody against somatic antigen (presumably principally nucleoprotein) also occurs with an age distribution similar to that of serum bactericidal activity against type b *H. influenzae* (7). The inverse relationship between serum bactericidal activity and the incidence of type b *H. influenzae* meningitis in the young child suggests a causal relationship between antibody and protection (8), but does not exclude an ancillary role for cell-mediated immunity.

For some time it has been recognized that somatic bacterial nucleoprotein antigens induce principally delayed-type cutaneous reactions whereas polysaccharide bacterial fractions result in the immediate-type cutaneous reaction in man (1). Specificity of cell-mediated immune reactions resides with the lymphocyte. Lymphocytes which are not adherent to fibers are apparently of the thymus-derived or T type (9). Lymphocytes in this study were purified by elution from cotton wool containing columns, and so it is assumed that their *in vitro* reactivity represents primarily T-lymphocyte response.

Protein-containing extracts of various bacteria yield high levels of *in vitro* transformation when the lymphocyte donor has a high level of delayed-type skin reactivity to the sensitizing antigen (10). Lower order, apparently nonspecific transformation due to bacterial endotoxin is apparently another type of lymphocyte reaction (10) which has a flat dose–response curve (3). In the present study both the somatic and polysaccharide antigens were prepared virtually free of endotoxin. Even if traces of endotoxin-like activity remained in the somatic antigen, the amounts present would have been far below that causing significant transformation.

Polysaccharide was a weak stimulant of blastogenesis in the present system, though *in vitro* transformation by higher concentrations may occur. However, the polysaccharide is an excellent antibody inducer in both adults and children (6) presumably by activation of B-lymphocytes which subsequently proliferate into specific antibody-producing plasma cells. Somatic antigen is the *H. in-*

fluenzae component which elicits the T-lymphocyte blastogenic response *in vitro,* and therefore, presumably is the bacterial principle which chiefly effects delayed hypersensitivity *in vivo.*

The high degree *in vitro* blastogenesis of lymphocytes from normal adults may result from active *in vivo* sensitization by bacterial components during *H. influenzae* infections. Alternatively, such reactivity may be acquired nonspecifically due to sensitization by cross-reactive bacterial antigens which are widespread among bacteria (11). Cross-reacting bacterial polysaccharides are suspected of causing in part the natural induction of antibody against *H. influenzae* polyribose phosphate(12).

Lymphocyte transformation caused by antigens from bacteria causing predominantly intracellular infections has a role in activating cellular immune responses which are protective in animals and function similarly in man (13). A role for cell-mediated immunity or delayed hypersensitivity in protection from *extracellular* bacterial pathogens is less certain and may be of only secondary importance after antibody-mediated immunity has become well established. However, very active cellular immunity to *H. influenzae* somatic antigen, exists as indicated by *in vitro* lymphocyte transformation. Perhaps lymphocyte directed cell-mediated immunity provides an ancillary defense *in vivo* against invasive *H. influenzae* infection. Since *H. influenzae* may flourish in the respiratory tract despite systemic antibody (14) lymphocytes sensitized to *H. influenzae* somatic antigen could confer beneficial cellular immunity in these sites. Alternatively, *H. influenzae* somatic antigen-sensitized lymphocytes could contribute to the pathogenesis of chronic sinobronchopulmonary disease via lymphocytotoxin (15).

Summary. Somatic antigen prepared from either nontypable nonencapulated or type b encapsulated *H. influenzae* strains is effective in low concentration in causing transformation *in vitro* of nonfiber-adherent human lymphocytes. Capsular *H. influenzae* polyribose phosphate is an ineffective stimulant for human lymphocyte transformation *in vitro.* Significant transformation of peripheral blood lymphocytes from all normal adults tested was effected by the somatic antigen.

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