Polyclonal Response of Human Lymphocytes to
Chlamydia trachomatis

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The mitogenicity of Chlamydia trachomatis was investigated. Umbilical cord blood cells from healthy newborns were used. These cells were stimulated in cultures with partially purified C. trachomatis elementary bodies. Proliferation of cultured lymphocytes and secreted immunoglobulins and leukocyte migration inhibitory factor were measured in the culture supernatants. Our results showed that C. trachomatis is able to elicit polyclonal antibody, lymphokine, and DNA synthesis.

MATERIALS AND METHODS

C. trachomatis preparation. The LGV strain (L2/434/Bu) grown in mouse epithelial cells (MMC-E) was kindly supplied by J. Keski-Oja (Labsystems; Helsinki, Finland). The cells were sonicated, and cell debris was removed by centrifugation at 200 × g for 10 min. Thereafter, chlamydial particles were sedimented by centrifugation at 20,000 × g for 60 min. The crude elementary-body preparation used contained about 10^9 inclusion-forming units/ml. For the control, similarly treated, mock-infected epithelial cells were used.

Cell isolation and cultures. Umbilical cord blood cells were used to test the possible mitogenicity of C. trachomatis. Cord blood cells were obtained from healthy newborns immediately after birth. The blood was centrifuged by Ficoll-Isopaque to obtain mononuclear cells (MNC). These cells contained, on the average, 84% lymphocytes and 16% monocytes. Monocytes were demonstrated by staining nonspecific esterase (22). For some experiments, monocyte contamination of lymphocyte suspensions was reduced by allowing monocytes to adhere to plastic surfaces, which diminished monocyte contamination to about 5%. Nontreated MNC and MNC partially depleted of adherent cells were used in cultures. For leukocyte migration inhibitory factor (LIF) and lymphocyte transformation experiments, the cell concentration was adjusted to 0.25 × 10^6/ml in 10% autologous plasma-RPMI 1640. In antibody synthesis experiments, the cell concentration was 2 × 10^6/ml and RPMI was supplemented with 10% fetal calf serum (Flow Laboratories, Irvine, Scotland). Samples (0.1 ml) of cell suspensions and various dilutions of chlamydial and control preparations were pipetted into wells of flat-bottomed microplates.

Lymphocyte transformation and LIF assays. Sixteen hours before harvesting, 0.125 μCi of [3H]thymidine ([3H]-thymidine) was added per well. The crude LIF and the three controls were incubated for 16 h, and the cultures were harvested. The cultures were centrifuged at 1,200 rpm for 10 min. The supernatants were harvested using a sterile pipette and the cells were washed with distilled water. Cell counts were made using a hemocytometer and the cells were dispersed in a cell counter (2). The supernatants were counted in a liquid scintillation counter (2).

Enzyme-linked immunosorbent assays. Immunoglobulin G (IgG) class antibodies to C. trachomatis in umbilical cord blood were determined by a commercial kit (Orion Diagnostica, Helsinki, Finland). None of the 25 umbilical cord blood samples tested contained measurable amounts of antichlamydial antibodies.

A double-sandwich enzyme-linked immunosorbent assay was used to measure IgM, IgG, and IgA levels in lymphocyte culture supernatants. Microtiter plates (Nunc, Roskilde, Denmark) were coated with rabbit anti-human IgM, IgG, or IgA antiserum (Dakopatts a/s). The antisera were diluted 1/1,000 (1/500 for IgA) in 0.01 M phosphate buffer (pH 7.5). The plates were incubated at 4°C overnight and then washed three times with phosphate-buffered saline containing 0.5% Tween 20 followed by three rinses with distilled water. Lymphocyte culture supernatants and human IgM (Dako Co., Santa Barbara, Calif.), IgG, and IgA (Sigma) for standard curves were diluted in 1% bovine serum albumin-0.1% Tween 20-phosphate-buffered saline and incubated for 30 min at 37°C. After a washing step, peroxidase-conjugated rabbit anti-human IgG, IgM, and IgA antibodies (Dakopatts a/s) diluted 1/400, 1/200, and 1/100, respectively, in 5% horse serum-0.1% Tween 20-phosphate-buffered saline were added to the plates. The plates were incubated for 1 h at 37°C and washed, after which the substrate (0.04% 1,2-phenylenediamine dihydrochloride [Fluka] and 0.15% hydrogen peroxide in 0.1 M citric acid-phosphate buffer [pH 5.0]) was

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added. The plates were incubated with the substrate at 37°C for 30 min. The enzymatic reaction was stopped by adding 2 M sulfuric acid, and the A492 was measured with a Titertek multiscan plate reader (Labsystems).

RESULTS

Stimulation of neonate lymphocyte proliferation by C. trachomatis. Neonate cells exhibit a high level of spontaneous proliferation, which may mask possible external-stimulant-induced activation. Therefore, culture conditions were determined which would reduce spontaneous proliferation. Incubating the cells at a relatively low density of $2.5 \times 10^6$ per well of flat-bottomed microplates diminished spontaneous proliferation while permitting detectable C. trachomatis-induced stimulation to occur. In preliminary experiments, we also found that there was no difference between MNC and iron-treated MNC (average monocyte contaminations, 16 and 5%, respectively). When mitomycin-treated MNC were cultured in the presence of C. trachomatis elementary bodies, no incorporation of labeled 5-iodo-2'-deoxyuridine took place (data not shown). Therefore, it is unlikely that chlamydiae themselves incorporated the label in stimulated cultures. A dilution of 1/100 of the chlamydial stock preparation produced optimal DNA synthesis (data not shown). Optimal incubation time for C. trachomatis-induced proliferation was 7 days, and mock-infected cell extract did not induce significant proliferation of the cells (Fig. 1).

Production of LIF by C. trachomatis-stimulated cells. The amounts of monocytes in neonate MNC were not inhibitory of C. trachomatis-induced LIF production (data not shown), and no effort was made to remove monocytes in these experiments. The peak LIF activity was detected in 3-day culture supernatants at a dilution of 1/100 of the chlamydial stock preparation (Fig. 2). Control cell extract did not induce significant LIF production (Fig. 2). LIF activity was lost partially at 56°C and totally at 80°C (data not shown). Also, a serine esterase inhibitor, phenylmethylsulfonfluoride, partially blocked LIF activity (data not shown).

Secretion of immunoglobulins in C. trachomatis-stimulated neonatal cell cultures. To find optimal conditions for neonate cell antibody formation, we tested several variables. Careful selection of fetal calf serum and the use of crowded culture conditions ($2 \times 10^6$ cells per well) appeared to be important. Adding 2-mercaptoethanol or feeding the cultures seemed to have no beneficial effect on antibody synthesis. MNC partially depleted of monocytes by plastic adherence clearly secreted more IgM than nontreated MNC (data not shown). Thus, in contrast with transformation and LIF experiments, it was necessary to reduce the amount of monocytes for optimal antibody production.

Nine-day culture supernatants contained slightly higher IgM levels than did 7-day cultures (Fig. 3). However, for practical reasons, the cells were incubated for 7 days in subsequent experiments. Elementary-body preparations diluted 1/100 produced peak IgM levels (data not shown). With all culture times and stimulant concentrations tested, levels of IgG and IgA in the supernatants were negligible (data not shown). Control cell extract did not induce antibody formation (Fig. 3).

DISCUSSION

We investigated the mitogenic effect of C. trachomatis on umbilical cord blood lymphocytes. Since neonate cells lack previous contact with antigens they can be used to study the mitogenicity of this organism. We found that C. trachomatis caused umbilical cord blood cells to proliferate and produce lymphokines and antibodies. We also found that amounts of monocytes that were inhibitory for antibody formation did not suppress proliferation or lymphokine production. This may reflect the presence of distinct cell populations that are responsible for different functions, as suggested by Gronowitz and Coutinho (10). In our hands, the mitogenicity of C.
trachomatis was relatively weak and of the same magnitude as that of lipopolysaccharide.

There are only a few earlier reports on C. trachomatis-induced in vitro lymphocyte activation, and the results of these studies are contradictory. C. trachomatis has been observed to induce interferon production and transformation (8) or transformation only (6) in seropositive individuals. According to Qvigstad et al. (18) C. trachomatis acts as a T-cell antigen and does not induce proliferation of neonate T cells. In contrast, Bard and Levitt (3) showed that chlamydial stimulate B cells to proliferate and secrete polyclonal antibodies. Evidently C. trachomatis can behave as both an antigen and a mitogen. Perhaps the high rate of spontaneous proliferation of neonate cells is one reason why a proliferative response has not been shown in prior studies. In our hands, significant C. trachomatis-induced proliferation was detectable after spontaneous proliferation was reduced by using low cell densities. Bard and Levitt (3) found that C. trachomatis did not induce proliferation of T cells contaminated with <1% OKM1 positive cells, i.e., monocytes. One may speculate whether T lymphocytes supplemented with 5 to 10% accessory cells would have responded.

We used the L2 serovar of C. trachomatis in the present study. This strain attaches in vitro to a great portion of peripheral blood lymphocytes and monocytes, yet it does not grow in them (4). Even if other serovars may interact in different ways with MNC, it is likely that common structures on the chlamydial surface, such as major outer membrane protein (7, 20) and lipopolysaccharide (15), induce a similar polyclonal response in lymphocytes.

The mitogenicity of chlamydial might explain certain clinical features of chlamydial infections. Mitogens are able to activate cells to produce a variety of antibodies, including autoantibodies. Autoantibodies have been detected in patients with chlamydial salpingitis (1) and lymphogranuloma venereum (13). Infants with chlamydial pneumonia have increased peripheral blood B lymphocytes and plasma cells and hyperimmunoglobulinemia (14). Histologically, dense infiltrations of plasma cells and lymphoid follicles (germinal centers) are characteristic for chlamydial cervicitis and endometritis (16, 17, 21). Autoantibodies, hypergammaglobulinemia, B lymphocytosis, and plasmacytosis all suggest that C. trachomatis is also a polyclonal B-lymphocyte activator in vivo.

In the present study, mitogenic properties of C. trachomatis were investigated by three different in vitro assays. Demonstration of mitogenicity of C. trachomatis serves to explain some specific features of acute and chronic chlamydial infections.

LITERATURE CITED
