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In Vitro Expression of Immunoglobulin M and G Subclasses by Murine B Lymphocytes in Response to a Polyclonal Activator from *Actinomyces*

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A cell wall extract from the gram-positive bacterium *Actinomyces viscosus* contains the mitogen AVIS, a potent polyclonal B-cell activator for murine B lymphocytes. Cultures of splenocytes from heterozygous nude mice in the presence of an optimal concentration of AVIS responded by a deoxyribonucleic acid synthesis response, and proliferaction reached maximal levels after 3 to 4 days. There was no requirement for T cells in the deoxyribonucleic acid synthesis, proliferactive, immunoglobulin M (IgM), or IgG responses. Significant numbers of IgM-producing cells were present as early as day 2 of culture, whereas later in the culture periods (days 3 to 6) IgG-producing plasmablasts and plasma cell were observed. In cultures of splenocytes from nude mice stimulated with AVIS for 4 to 5 days, 20 to 25% of the recoverable cells synthesized IgM, and 10% contained only IgG2 or IgG3; 5 to 8% of the cells stained for both IgM and IgG2 or both IgM and IgG3. Fine-structure analysis of AVIS-stimulated splenocytes from heterozygous nude mice after 3 days of culture demonstrated that 20 to 25% of the cells were activated to various degrees. Of most importance, all of the activated cells had the characteristic of B lymphoblasts, plasmablasts, or plasma cells. This is the first demonstration of a polyclonal B-cell activator other than lipopolysaccharide which induces IgG3 synthesis. We suggest that AVIS may be a useful probe for the exploration of the functional activities of subpopulations of B cells.

A great deal of knowledge of the molecular and cellular events occurring after B-lymphocyte activation has been gained through the use of polyclonal B-cell activators. Lipopolysaccharide (LPS) from gram-negative bacteria (12, 17, 18, 23, 24), dextran sulfate (12, 14), purified protein derivative (26), and other polyclonal B-cell activators (PBAs) (2, 9, 22) have been useful tools for the exploration of B-cell activation and differentiaton. In particular, LPS stimulation can initiate immunoglobulin M (IgM), IgG1, IgG2, and IgA synthesis by cultured cells from mouse thoracic duct, spleen, lymph node, Peyer's patches, and bone marrow (17, 19).

From these studies has evolved the attractive hypothesis that the molecular and cellular events occurring after polyclonal activation are comparable to the events occurring after specific-antigen activation. However, there are shortcomings to this hypothesis since only B-cell activation by LPS has been well characterized. In addition, B lymphocytes of adult mice are a heterogeneous collection of cells. Gronowicz and Coutinho (13, 15) have shown that several different PBAs can induce proliferation or immunoglobulin synthesis or both. However, each polyclonal B-cell activator selectively activates a distinct subset of B cells, which are believed to represent different but successive stages along a maturational pathway. Our knowledge of phenotypic characteristics of these B-cell subsets and of the steps and sequence in B-cell maturation may be greatly augmented by the use of additional polyclonal B-cell mitogens.

In a previous report (10), we have demonstrated that a cell wall mitogen (AVIS) from the gram-positive bacterium *Actinomyces viscosus* activates murine B cells to deoxyribonucleic acid (DNA) synthesis and proliferation in the absence of T cells. Other investigators (5) have reported that a heteroglycan from *A. viscosus* induced proliferation in splenocytes and thoracic duct cells of rats. In addition, spleen cells were stimulated in vitro to develop into direct plaque-forming cells against trinitrophenol-sheep erythrocytes.

We have done experiments aimed at answering the following questions relevant to B-cell activation and differentiation. (i) Can IgM, IgA, and particulary IgG syntheses be initiated by AVIS? (ii) If so, what subclasses of IgG are synthesized? (iii) Can mature plasma cells be

formed in vitro in response to AVIS? (iv) What are the fine structural features of the AVIS-activated cells? (v) Are T cells required for the IgG and IgA synthesis response?

MATERIALS AND METHODS

Mice. Congenitally athymic nude mice (*nu/nu*) on a C57BL/6J background and their heterozygous (*nu/*+) littermates were provided by Elliot Parks, Scripps Clinic and Research Foundation, La Jolla, Calif. Mice used were 6 to 16 weeks of age and were of only one sex in any given experiment.

Preparation of AVIS mitogen. *A. viscosus* bacteria (strain WVU/371) were grown in brain heart infusion medium and disrupted in a Braun glass bead homogenizer as previously described (10). The homogenate was heated with hot 10% trichloroacetic acid for 30 min and washed exhaustively with distilled water to obtain a partially purified cell wall fraction (26). Preliminary experiments revealed that this material contained all of the mitogenic activity originally observed in the crude homogenate. Because the trichloroacetic acid-purified AVIS is highly insoluble, it was suspended in a 0.1 M phosphate buffer, pH 7.0, containing 1% sodium dodecyl sulfate and 0.1 M 2-mercaptoethanol and heated for 10 min at 100°C. The sodium dodecyl sulfate was removed by passing the AVIS over a column (0.5 by 20 cm) of AG 2-X10 resin equilibrated in pH 7.0 phosphate buffer (20). The column effluent was dialyzed against distilled water for 48 h to remove the 2-mercaptoethanol, lyophilized, and stored over $CaCl_2$ at −70°C. The AVIS material thus prepared is soluble in cell culture medium and is a very potent murine B-cell mitogen, routinely giving DNA synthesis stimulation indices of 50 to 200.

Lymphocyte preparation and culture conditions. Spleen cells were teased from the capsule and suspended in RPMI 1640 medium (GIBCO, Laboratories, Grand Island, N.Y.) containing penicillin (100 U/ml), streptomycin (100 μg/ml), 4 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), and 5×10^{-5} M 2-mercaptoethanol. Aggregates of cells were dispersed by pipetting, and any remaining large tissue pieces were allowed to settle out at $1 \times g$ for 3 min. The cells were decanted, pelleted at $150 \times g$ for 10 min, suspended briefly in 0.83% (wt/vol) ammonium chloride solution to lyse erythrocytes and repelleted. The cells were suspended in medium containing 5% (vol/vol) heat-inactivated (56°C for 30 min) human serum, and 1-ml samples containing 5×10^5 viable cells per ml were dispensed into Falcon 3033 tubes. AVIS was added to some cultures in 100 μl of RPMI 1640 medium, and the cultures were incubated at 37°C in 5% CO_2–95% air.

Measurement of DNA synthesis. After the appropriate culture period, cells were incubated for an additional 4 to 6 h with 0.5 μCi of [^{125}I]deoxyuridine (specific activity, >200 Ci/mmol; New England Nuclear Corp., Boston, Mass.) and 10^{-6} M 5-fluorodeoxyuridine (Aldrich Chemical Co., Milwaukee, Wis.) (1). At the end of the experimental period, cells were pelleted at $150 \times g$ for 10 min, washed once with Hanks balanced salt solution, and treated with cold 5% (wt/vol) trichloroacetic acid; the precipitate was pelleted at $1{,}000 \times g$ for 10 min. The radioactivity of the precipitates was determined in an automated gamma counter.

Staining for cytoplasmic immunoglobulin. Lymphocyte cultures were harvested and washed twice in cold medium. Cells were deposited onto glass slides, briefly air dried, and fixed for 10 min in cold 95% ethanol. The slides were washed thoroughly in phosphate-buffered saline, pH 7.2, and stained immediately. Smears were incubated with one or more class-specific goat anti-mouse conjugates, whose concentrations were previously determined to be optimal, in a moist chamber for 30 min at room temperature. The slides were washed three times in phosphate-buffered saline and mounted in 90% buffered glycerol, pH 7.0.

Antibodies and conjugates. Antisera were raised in goats with the myeloma proteins MOPC 104E (μ, λ), MOPC 315 (α, κ), and normal mouse 7S IgG. IgG fractions were obtained from these sera by $(NH_4)_2SO_4$ precipitation and DEAE 52-cellulose chromatography and were absorbed to class specificity with myeloma proteins coupled to Sepharose beads. Fluorescein isothiocyanate (FITC) obtained from BBL Microbiology Systems, Cockeysville, Md., was added at a ratio of 1 mg of FITC to 20 mg of immunoglobulin dissolved in pH 9.6 carbonate buffer. The conjugation was allowed to proceed for 12 to 18 h at 6°C, and the unreacted fluorochrome was removed by passage over a Bio-Gel P-2 column equilibrated in phosphate-buffered saline. The resulting conjugates were applied to small DEAE 52-cellulose columns equilibrated in 0.01 M phosphate buffer, pH 6.8, and eluted with 0.01 M phosphate buffers containing 0.05, 0.1, 0.2, and 0.5 M NaCl. Fractions having molar FITC-to-protein ratios of 1.5 to 3 were used for staining. The specificity of the FITC conjugates was determined by performance testing against Sepharose beads coupled with MOPC 104E (μ, λ), MOPC 315 (α, κ), UPC 10 (IgG2a, κ), MOPC 195 (IgG2b, κ), MOPC 21 (IgG1, κ), FLOPC 21 (IgG3, κ), MOPC 41 (κ), and RPC 20 (λ), as well as a panel of fixed myeloma cells. Staining was detected on only Sepharose beads and fixed myeloma cells of the expected class. Tetramethylrhodamine isothiocyanate (RITC) antibodies highly specific for the mouse subclasses IgG1, IgG2, and IgG3 and whose specificity has been previously described were kindly provided by J. F. Kearney, University of Alabama, Birmingham (17–19).

Fluorescence microscopy. A Zeiss fluorescence microscope with transmitted light, a dark-field condenser, and a 200-W high-pressure mercury bulb was equipped with KP-546 and KP-500 excitation filters (Carl Zeiss, Inc.) coupled with appropriate barrier filters to detect the fluorescence of rhodamine and fluorescein, respectively (8). Photographs were made with Kodak Ektachrome film.

In all experiments, cells were counted under dark-field illumination to enumerate the total number of cells per field followed by the appropriate filtration to visualize the fluorochrome-stained cells. A total of 2,000 cells were counted for each preparation.

Electron microscopy. Mitogen-stimulated and nonstimulated control splenocytes were prepared for electron microscopy after 3 days of culture. After fixation for 2 min in a 1:1 mixture of RPMI 1640

medium and freshly prepared 2% glutaraldehyde buffered with 0.2 M sodium cacodylate (pH 7.4), the cells were transferred to centrifuge tubes with conical bottoms. The cells were pelleted by centrifugation at 150 × *g* for 10 min, the supernatant was removed, and 2 ml of fresh 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) was added. The cells were fixed for 1 h, washed three times with buffer, and postfixed with 1% OsO_4 in 0.1 M cacodylate buffer for 1 to 2 h.

The cells were then washed twice with distilled water, and the pelleted cells were stained en block in 0.5% uranyl acetate for 10 min. After a distilled water rinse, the cell pellets were embedded in 1% agar and cut into 1 to 2 mm^2 cubes. Dehydration was done by a graded series of ethanol, and the cubes were embedded in Epon 812 (21). Ultrathin sections were made with an MT-1 microtome with a diamond knife, stained with uranyl acetate and lead citrate (11), and viewed and photographed with a JEOL 100B electron microscope.

Autoradiography. After the appropriate culture period in the presence or absence of AVIS, the cells were labeled with 0.1 μCi of [*methyl*-^{3}H]thymidine (6.7 Ci/mmol; New England Nuclear Corp.) for an additional 24 h. The cells were washed, layered onto fetal calf serum, and centrifuged at 200 × *g* for 10 min. After resuspension, the cells were smeared onto slides, air dried, fixed, and processed for autoradiography (7). Sets of slides were developed after various periods of time to ensure the detection of all cells incorporating the radioactive DNA precursor. The slides were stained with McNeal tetrachrome stain, cover slips were placed on the slides, and 1,000 cells were counted in each preparation. Cells with a diameter of 10 μm or larger were designated as blast cells; more than 98% of these cells had incorporated [*methyl*-^{3}H]thymidine.

RESULTS

AVIS-induced proliferation and syntheses of IgM and IgG in splenocytes from heterozygous nude (*nu*/+) mice. Pilot experiments revealed that 5 × 10^5 splenocytes per ml of culture medium and 50 to 100 μg of AVIS per ml provided optimal conditions for immunoglobulin production and proliferation. AVIS induced a time-dependent increase in DNA synthesis (stimulation index, 50) which reached maximal values during days 3 and 4 of culture (Fig. 1). By autoradiography, 20 to 25% of the recoverable cells from AVIS-stimulated cultures incorporated [*methyl*-^{3}H]thymidine and had a blast cell morphology on day 4 of culture (data not shown). Paralleling the increase of DNA synthesis was a fivefold increase in the number of viable cells. In the control cultures, there was no increase in cell numbers or DNA synthesis during the 6-day culture period.

The peak day of culture for the appearance of cytoplasmic IgM (c-IgM)-containing cells occured 1 to 2 days later than the optimal days of DNA synthesis (day 5 versus days 3 to 4) (Fig. 2). There was an initial lag of 3 days before a significant expression of c-IgG was detected. At the same time (day 3), the number of c-IgM-bearing cells was sixfold higher than the number of c-IgG-positive cells (12 versus 2%). Maximal numbers of c-IgG-containing cells were observed on days 5 and 6 of culture but were only half as numerous as c-IgM-containing cells. In control cultures in which no AVIS was added, the spontaneous induction of c-IgM or c-IgG never exceeded 1% of the recoverable cells.

AVIS-induced DNA synthesis and IgG and IgM production in splenocytes of homozygous nude (*nu/nu*) mice. Kearney and Lawton (18) have reported that LPS stimulates IgM production in vitro and that with time a portion of these cells also make IgG1 and IgG2. These events have been shown to be T independent (17, 18). To determine whether AVIS induces a similar response, AVIS-stimulated splenocytes from T-deficient (congenitally athymic) C57BL/6J mice were examined for the magnitude of DNA synthesis response or simultaneous expression of either IgG1, or IgG2 or IgG3 and IgM, as well as for the singular expression of each major class of immunoglobulin.

The overall pattern of DNA synthesis of T-deficient splenocyte cultures activated by AVIS was similar to that observed in cultures of cells from heterozygous littermates. The peak of DNA synthesis as measured by [^{125}I]deoxyuridine incorporation reached a maximal level by day 3 of culture (Fig. 3). The frequency of cells with blast cell morphology was highest (12 to 20% of the cells) on day 3 of culture, as expected from the [^{125}I]deoxyuridine data. The frequency of blast cells, more than 98% of which had incorporated [*methyl*-^{3}H]thymidine, declined to 50% of the peak numbers during the remainder of the experiment (data not shown).

The percentage of cells with only c-IgM reached a peak of approximately 20% of the total recoverable cells of day 5 of culture (Fig. 4A). As shown in Fig. 4B, an additional 10% of the recoverable cells produced either c-IgG2 or c-IgG3 exclusively. The frequencies of c-IgG2 and c-IgG3 cells were approximately equivalent throughout the culture period and for this reason are presented together. However, Fig. 4C shows that a further 5 to 8% of the total recoverable cells containing c-IgM after days 4 to 6 of culture expressed c-IgG_2 or c-IgG_3 simultaneously. Minor differences in the temporal appearance of IgG-synthesizing cells were noted between cultures of nude (Fig. 4) and heterozygous (Fig. 2) splenocytes.

Cells containing c-IgG1 and c-IgA were observed infrequently (0.9 and 1.0%, respectively) and had a blast cell morphology when present. Without the addition of AVIS, no detectable

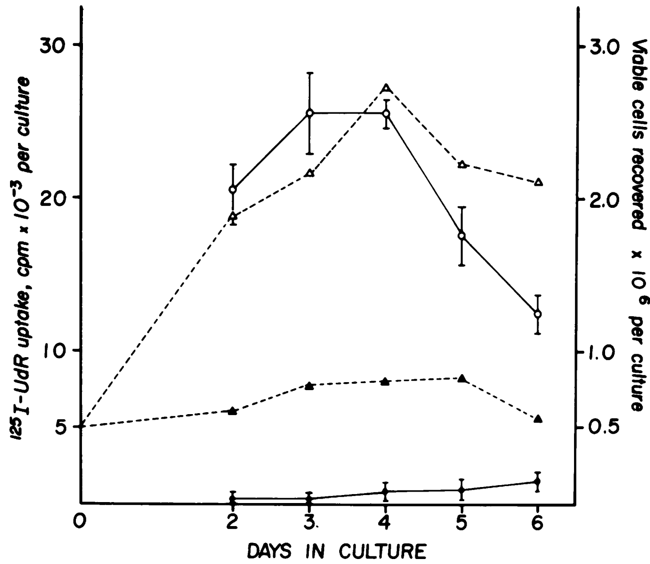


FIG. 1. *Kinetics of [^{125}I]deoxyuridine incorporation and cell proliferation in AVIS-stimulated and control cultures of C57BL/6J heterozygous nude splenocytes. The scale on the left ordinate shows [^{125}I]deoxyuridine incorporation of AVIS-stimulated* (○) *and control* (●) *cultures. Data are presented as mean counts per minute ± 1 standard deviation. The scale on the right ordinate shows cell recovery in AVIS-stimulated* (△) *and control* (▲) *cultures.*

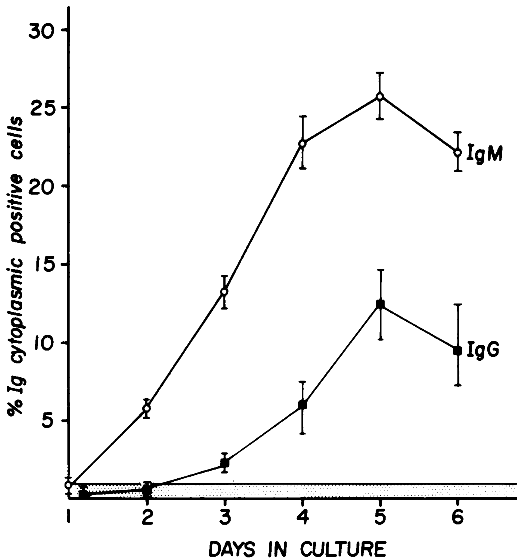


FIG. 2. *Kinetics of appearance of c-IgM-positive* (○) *and IgG-positive* (■) *cells in AVIS-stimulated cultures of C57BL/6J heterozygous nude splenocytes. The level of IgM- and IgG-positive cells in control cultures is indicated by the shaded area. Data are presented as mean ± 1 standard deviation.*

increases in c-IgM, c-IgG2, or c-IgG3 cells were observed during the 6 days of culture (Fig. 4A, B, and C).

After AVIS stimulation, the morphology of cells containing cytoplasmic immunoglobulin was heterogeneous. Large blast cells predominated early (days 2 to 3), whereas cells with a mature plasma cell morphology appeared from days 3 to 6 of culture (Fig 5). Fluorescence photomicrographs of AVIS-activated cells producing both c-IgM and c-IgG2 or c-IgG3 are shown in Fig. 6.

Fine structure of AVIS-responding cells. Electron microscopic examination of the ultrastructural features of AVIS and control cells was done after 3 days of culture of T-sufficient (heterozygous) splenocytes. Cells from the unstimulated cultures were predominantly small or medium lymphocytes with fewer numbers of macrophages. The lymphocytes had densely clumped heterochromatin, sparse rough endoplasmic reticulum, and a high nucleus-to-cytoplasm ratio (Fig. 7A). In contrast, after 3 days of incubation of AVIS, 15 to 25% of the cells had a morphology consistent with activated B lymphocytes. Plasmablasts and plasmacytoid cells comprised the bulk of the activated cells (Fig. 7B). These cells showed extensive rough endoplasmic reticulum, which was often dilated. Many of the plasmacytoid cells had myelin inclusions, vacuoles, and well-developed Golgi zones.

DISCUSSION

In this paper, we have investigated the behavior of and the products made by B cells activated by the mitogen AVIS from the gram-positive bacterium *A. viscosus*. Specifically, the data pre-

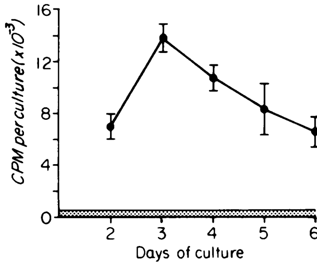


FIG. 3. *Kinetics of [^{125}I]deoxyuridine incorporation in AVIS-stimulated cultures of splenocytes from homozygous nude C57BL/6J mice. Data are presented as mean ± 1 standard deviation. The level of [^{125}I]deoxyuridine incorporation in control cultures is indicated by the shaded area.*

sented in this report demonstrate that splenic B cells were activated by AVIS to proliferate and produce immunoglobulin. Proliferation preceded the development of maximal levels of IgM- and IgG-producing blast cells by days 1 to 2. Large numbers of IgM- and IgG-producing plasma cells were found after days 4 to 5 of culture. When T-deficient splenocytes from homozygous nude mice were cultured with AVIS, the overall pattern of proliferation and immunoglobulin responsiveness was not different from that of cells from T-sufficient heterozygous littermates.

A portion of the precursor cells "recruited" by AVIS produced IgG3 and IgG2 subclasses of immunoglobulin. This recruitment was independent of T cells. To our knowledge, AVIS is the first polyclonal B-cell activator other than LPS (19) reported to induce the synthesis of IgG3. There are several possible explanations for this novel finding. First, AVIS may activate precursors of either IgG1 or IgG2 to switch to IgG3 synthesis after activation. If this were the case, a wave of c-IgG1- or c-IgG2-staining lymphoblasts would precede the appearance of c-IgG3-synthesizing cells. This possibility is not likely since IgG1 synthesis was not observed at all and IgG2 and IgG3 appeared in approximately equal numbers at all time points examined. However, we cannot exclude the possibility that some of the precursors produced undetectable amounts of IgG1 and IgG2 before switching to IgG3 production.

Our operating hypothesis is that AVIS activates many discrete subpopulations of B cells to proliferate, divide, and produce immunoglobulin. Contained in one or more of these subsets are the precursor cells for IgG3. Our data suggest that most, if not all, of these cells probably produce IgM before or simultaneously with IgG3 (Fig. 4). Then, a portion of this subset may switch to exclusively IgG3. AVIS also activates a subset(s) that will produce IgM only and one or more other subsets that will first synthesize IgM followed by IgG2.

Our finding that optimal DNA synthesis preceded optimal immunoglubulin production by 1 to 2 days is in general agreement with the observations of other investigators who have used LPS (16–18), *Listeria* cell wall mitogen (9), a polysaccharide from *Serratia* (22), and *Nocardia* extracts (3, 4). In all of these cases, DNA synthesis and cell division preceded slightly or were simultaneous with plasma cell formation and immunoglobulin synthesis and secretion.

Other similarities between the LPS and AVIS polyclonal responses emerge when the magnitudes of the proliferative and immunoglobulin production responses are compared by enumerating the frequency of blast cells and cells with c-IgM and c-IgG. In the presence of AVIS, 10 to 30% of the recoverable cells during days 2 and 3 of culture were blast cells and had incorporated [*methyl*-^{3}H]thymidine (unpublished observa-

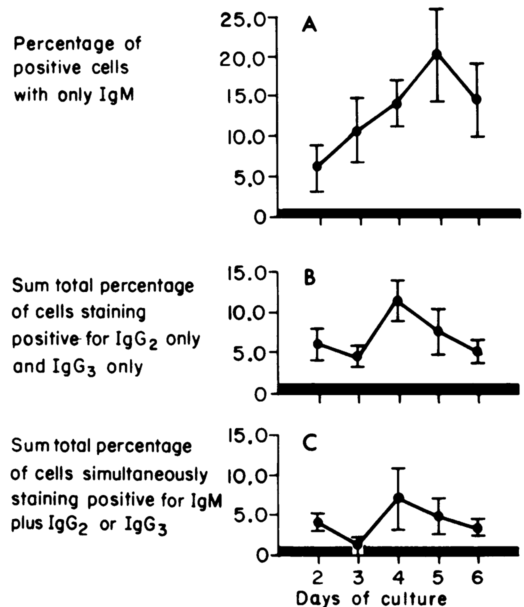


FIG. 4. *(A) Temporal appearance of cells synthesizing only IgM. (B) Appearance of cells synthesizing only IgG2 or only IgG3. The frequency of cells synthesizing only IgG2 and that of cells synthesizing only IgG3 were added together since these cells were present at approximately equal frequencies. (C) Appearance of cells synthesizing IgM simultaneously with IgG2 or IgG3. The frequency of cells synthesizing IgM plus IgG2 and that of cells synthesizing IgM plus IgG3 were added together since these cells were present at equal frequencies. The data for (A), (B), and (C) are shown as the mean ± 1 standard deviation, and the solid black area represents the mean background values ± 2 standard deviations.*

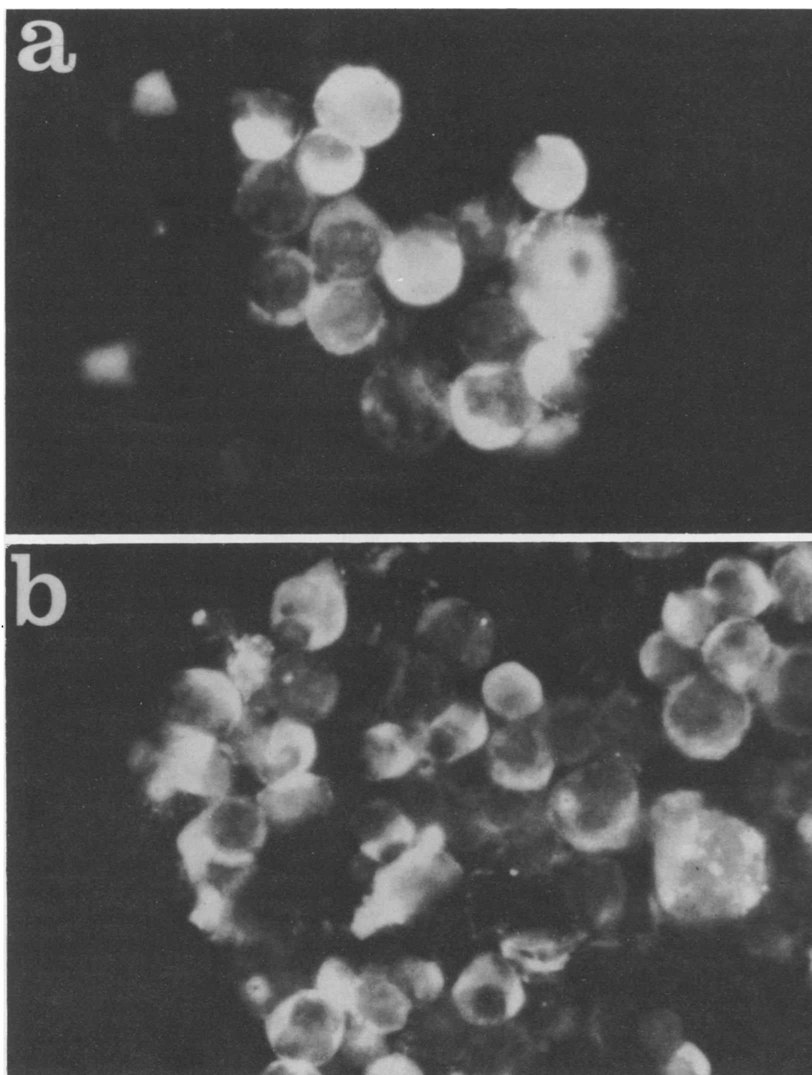


FIG. 5. *Fluorescence photomicrographs of AVIS-stimulated cultures. (a) Two-day culture of T-deficient splenocytes stained with fluorescein isothiocyanate anti-mouse IgM. Notice the numerous large blastlike cells containing cytoplasmic IgM. (b) Six-day culture of T-deficient splenocytes stained with fluorescein isothiocyanate anti-mouse IgM. Notice the many cells with plasmablast and plasma cell morphology. Original magnification,* × *1,000.*

tions). Janossy and Greaves (16) reported that percentages of splenocytes responsive to LPS were approximately 17 to 30% of the total recoverable cells. In both of these cases, one-fourth to one-half of all B cells responded by DNA synthesis and division. Furthermore, the numbers of IgM and IgG precursors that AVIS (Fig. 2 and 4) and LPS (16–18) stimulate are equivalent in both the presence and the absence of T cells. These findings therefore support the contention that AVIS is a polyclonal B-cell mitogen of approximately the same potency as LPS because it activates a similarly large proportion of splenic B lymphocytes to synthesize DNA and immunoglobulin.

T cells apparently play a small role, if any, in the inductive, proliferative, and maturational events generated by the interaction of AVIS with splenic B cells. Several pieces of evidence support this notion. In a previous report, AVIS was demonstrated not to stimulate thymocytes to proliferate, and the proliferative capacities of T-deficient (nude or anti-rat brain plus complement-treated) splenocyte populations were

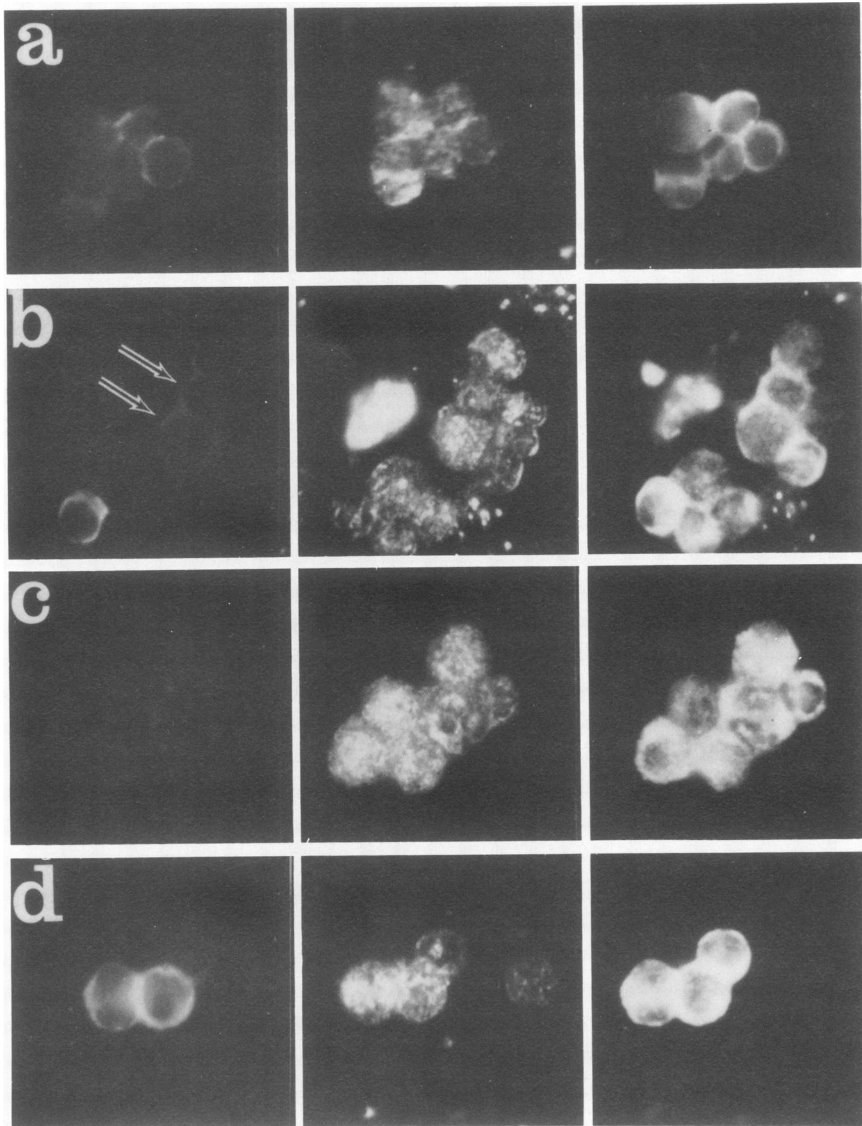
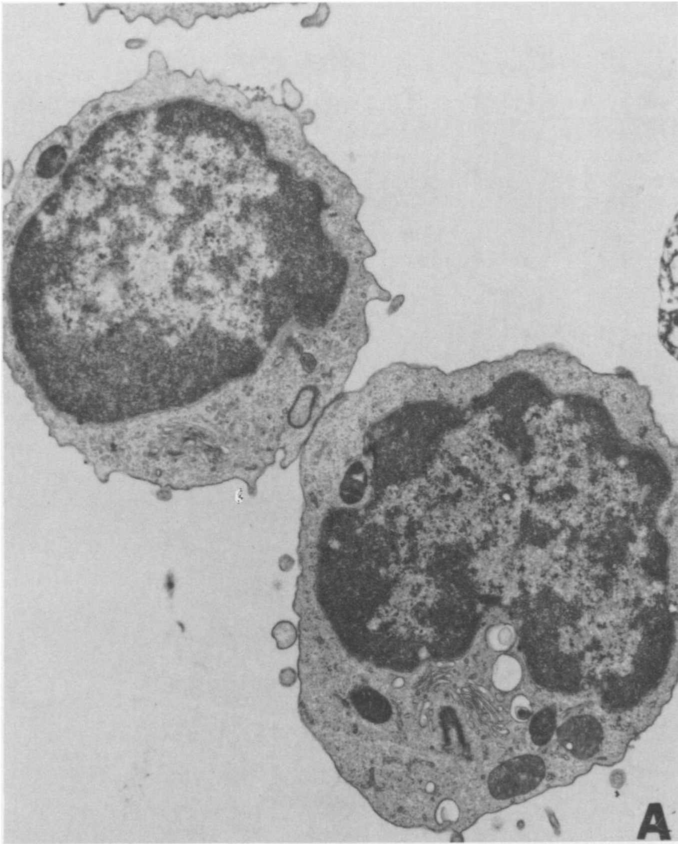


FIG. 6. *Fluorescence photomicrographs of AVIS-stimulated cells from homozygous nude mice after 4 days of culture. Cells were stained with mixtures of fluorescein- and rhodamine-conjugated antibodies. Each panel consists of the same field photographed under dark-field illumination (center) and selective filtration for rhodamine (left) and fluorescein (right). Original magnification, × 1,000. (a) RITC anti-IgG1 and FITC anti-IgM. Notice the apparent surface staining on two cells with RITC anti-IgG1 (left) and the same two cells stained for cytoplasmic IgM (right). (b) RITC anti-IgG2 and FITC anti-IgM. One plasmablast cell stained intensely for both IgG2 and IgM, whereas two others (arrows) stained lightly for IgG2 but intensely for IgM. (c) RITC anti-IgG3 and FITC anti-IgM. Five of six cells in this field stained intensely with FITC anti-IgM (right); the cells had synthesized little, if any, IgG3 (left). (d) RITC anti-IgG3 and FITC anti-IgM. Three of four cells stained intensely for mouse IgM (right), and two of the three simultaneously stained for IgG3 (left).*

FIG. 7. *Electron photomicrograph of control and AVIS-stimulated cells. (A) Ultrastructural appearance of cells from a 3-day culture in medium only. Notice the densely clumped heterochromatin and high nucleus-to-cytoplasm ratio. Original magnification, × 5,500. (B) AVIS-stimulated cells from a 3-day culture. These cells have ultrastructural features consistent with plasmablasts and plasma cells. Original magnification, × 5,500.*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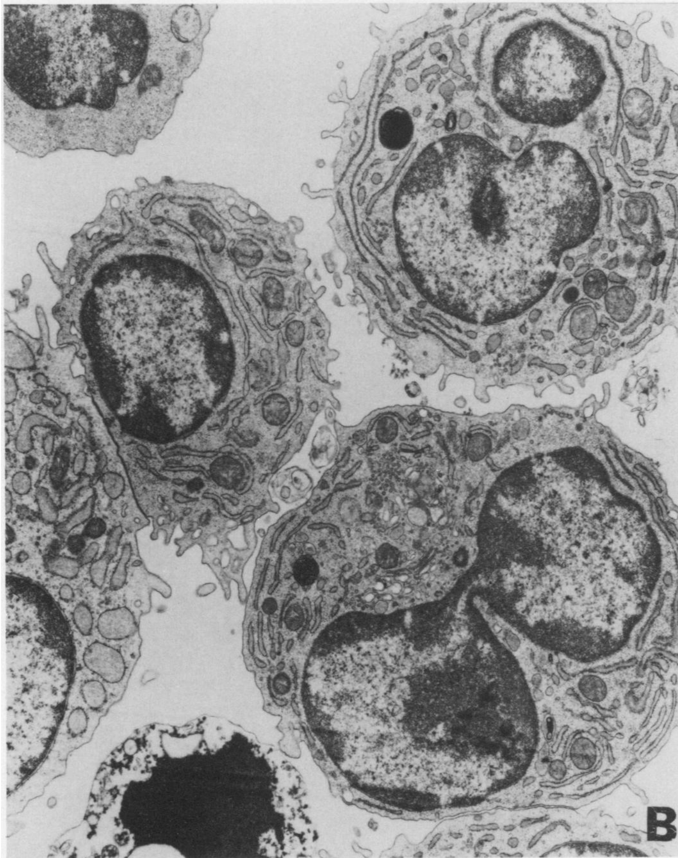
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equivalent to those of T-sufficient preparations (10). The fact that cells of the IgM and IgG classes were observed with the same or slightly increased frequencies in cultures of nude splenocytes strongly suggests that T-cell signals are not obligatory for in vitro polyclonal B-cell maturation. As determined by electron microscopic examination in this study, the ultrastructural features of cells cultured with AVIS and harvested at the peak of the DNA synthesis response were without exception those of activated B lymphoblasts or plasmacytoid cells. Added to our observations are those of Kearney and Lawton (18) and Kearney et al. (19) in which fetal or neonatal liver and spleen cells stimulated by LPS gave rise to plasma cells producing IgM, IgG1, IgG2, IgG3, and IgA at a time when thymus function is minimal. Taken in total, these observations suggest that B cells in the absence of T cells may be triggered by the appropriate polyclonal B-cell activator to express their full maturational potential.

Although it is unlikely, the possibility that T-cell precursors present in the nude splenocyte preparations matured during the early days of culture and exerted regulatory activities on proliferation and immunoglobulin production cannot be completely excluded. The observations of Scheid et al. (25) suggest that T-cell precursors from a number of organs can be induced by a variety of ligands to express cell surface markers (Thy 1.2) characteristic of mature T lymphocytes. Exactly what spectrum of regulatory activities can be manifested by such cells remains unclear.

The similarities in the magnitude of the DNA synthesis response, the temporal pattern of immunoglobulin production with the associated restriction of immunoglobulin class without the necessity for T cells, and the morphological shift from blasts to plasma cells initiated by AVIS suggest that the subpopulations of B cells activated by LPS and AVIS are highly overlapping. Indeed, by selective suicide techniques with bromodeoxyuridine, stimulation with either LPS or AVIS in the presence of bromodeoxyuridine removed all cells responsive to either polyclonal B-cell activator upon restimulation in vitro (unpublished observation). The similar behavior of B cells activated by either LPS or AVIS appears to be a highly integrated series of genetically preprogrammed events. The highly ordered expression of the genetic program of B cells may not be dependent on the activating ligand, but rather may be an intrinsic quality of the population of cells activated.

In a previous paper (6), we speculated that bacterium-derived polyclonal B-cell activators may play a role in the pathogenesis of certain long-term inflammatory diseases. One such disease is human periodontitis, which is characterized by a dense accumulation of plasma cells and a concomitant loss of collagenous gingival connective tissue and alveolar bone. *A. viscosus* is one of a number of bacterial pathogens associated with human periodontitis. Although we do not yet have definitive proof that AVIS is a polyclonal B-cell activator for human cells, preliminary findings strongly support this contention. Therefore, it is possible that the pathogenicity of *A. viscosus* may be due in part to its ability to activate large numbers of B lymphocytes to immunoglobulin and lymphokine production.

ACKNOWLEDGMENTS

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