Brief Definitive Report

VIRUSES DISRUPT FUNCTIONS OF HUMAN LYMPHOCYTES

II. Measles Virus Suppresses Antibody Production by Acting on B Lymphocytes

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The ability of viruses to suppress the host's immune system is a matter of both biomedical interest and clinical importance. Historically, measles was the first virus observed to clinically alter immune function (1). The virus is responsible for severe disease as a result of this property, which allows activation of other infectious agents (2). Although measles is now controlled in Western countries by vaccination, it remains a serious problem for populations in the Third World (3).

Measles virus disrupts several functions of lymphocytes in vivo, including the ability to express delayed hypersensitivity and to mount humoral responses (4). The virus infects monocytes, T lymphocytes of both helper and suppressor subsets, and B lymphocytes (5, 6). Although survival in vitro is not altered, infected lymphocytes fail to generate NK cell activity or synthesize Ig (7). The mechanism of suppression of these lymphocyte functions by measles virus is not known. Because the model of PWM-driven Ig secretion allows the experimenter to examine defects in B lymphocytes or in the collaborating T lymphocytes and monocytes (8), we chose to focus initially on measles virus suppression of this lymphocyte function. The questions addressed in this paper are two. Can measles virus–infected monocytes and T lymphocytes produce sufficient help, as T cell–conditioned media (TCM), to drive B lymphocytes to proliferate and to secrete Ig? Does measles virus infection of B lymphocytes directly alter their ability to produce Ig? In this report, we localize measles virus–induced suppression of Ig synthesis to a direct effect on the B lymphocyte.

Materials and Methods

Cell Preparations. PBMC were isolated from heparinized venous blood of normal donors by density centrifugation on Ficoll-diatrizoate (Histopaque-1077; Sigma Chemical

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Co., St. Louis, MO). Monocytes were depleted by adherence to glass Petri dishes coated with FCS (Flow Laboratories, Inc., McLean, VA) and recovered by aspiration in PBS. The nonadherent mononuclear cells were further depleted of monocytes by carbonyl-iron phagocytosis. T lymphocyte- and B lymphocyte-enriched populations were selected by rosetting with neuraminidase-treated sheep erythrocytes, as previously described (7). NK cells were separated from the B lymphocyte population using the mAbs, anti-Leu-7, and anti-Leu-11b (Becton Dickinson Immunocytometry Systems, Mountain View, CA) followed by a fluorescein-conjugated $F(ab')_2$ goat anti-mouse IgM (Cappel Laboratories, Cochranville, PA) and cell sorting (FACS IV, Becton Dickinson Immunocytometry Systems). Natural cytotoxicity was measured by lysis of K562 cells in a ^{51}chromium-release assay, as previously described (7).

Preparation of TCM. T lymphocytes, 2×10^6/ml, and monocytes, 5×10^4/ml, were cultured with PWM (Gibco Laboratories, Grand Island, NY), 2.5 μg/ml, in RPMI 1640 medium (Media Unit, Scripps Clinic and Research Foundation) supplemented with 10% FCS for 48 h. Culture supernatants were centrifuged at 100,000 *g* for 1 h, passed through a 0.2 micron filter, and frozen before use. This treatment reduced infectious measles virus to <20 PFUs/ml. PWM was added to control, unstimulated T cell-monocyte cultures just before harvesting the supernatant.

Culture Conditions and Measurement of Secreted Igs. 10^5 B lymphocytes per well were cultured in 0.3 ml final volume in 96-well flat-bottomed microwell plates (Costar, Cambridge, MA) in RPMI 1640 with 10% FCS. PWM (2.5 μg/ml) and 2-ME (5×10^{-5} M; Eastman Kodak Co., Rochester, NY), were present in all cultures. TCM were added to cultures at 50% final volume. Supernates of B lymphocyte cultures were harvested after 7 d of incubation, and total IgG and IgM were measured by an enzyme-linked immunosorbent assay as described (7). There was no detectable Ig in TCM supernants.

Measles Virus Infection. The Edmonston strain of measles virus, passaged and plaqued on Vero cells, was used to infect lymphocytes at a multiplicity of infection of three, as previously described (7). Virus stocks were not contaminated with mycoplasma as tested by Hoechst 33258 stain (Serva Feinbiochemica, Heidelberg, FRG) (9). Measles virus antigens were detected in infected lymphocytes by direct immunofluorescence using a rhodamine-conjugated IgG preparation from the serum of a patient with subacute sclerosing panencephalitis (5, 7).

Cell Viability and Proliferation Assays. To count viable B cells, cells in 100 μl were aspirated from triplicate culture wells, pooled, diluted in 0.1% Trypan Blue (Eastman Kodak Co.), and 100–200 cells were counted in a hemocytometer. To measure proliferation, cultures were pulsed with 1 μCi/ well [^{3}H]thymidine, (6.7 Ci/mmol, New England Nuclear, Boston MA) for 4 h, then cultures were harvested onto glass fiber filters and counted in a liquid scintillation beta counter (Searle Analytic, Des Plaines, IL).

Electron Microscopy. B lymphocytes were infected with measles virus and cultured in TCM with PWM for 48 h. $1–2 \times 10^7$ viable cells were pelleted and resuspended in cold, 2.5% glutaraldehyde in PBS, and processed for electron microscopy as previously described (10).

Results and Discussion

PWM, a polyclonal B cell activator, requires Th and monocytes to drive B cell differentiation, but this accessory cell requirement can be replaced by soluble factors from stimulated T cells (8). B cells were cultured with TCM from stimulated T cells and monocytes. Thus, either B cells or T cells could be infected separately, and infectious virus was cleared from TCM by ultracentrifugation. The results of six experiments using this procedure showed that virus infection of B cells, but not T cells and monocytes, mediates suppression of Ig synthesis in PWM cultures (Fig. 1). Mock-infected B cells, when cultured in TCM from stimulated T cells, secreted 10 times more IgG and 6 times more IgM than control cultures. TCM from infected T lymphocytes were often more stimulatory

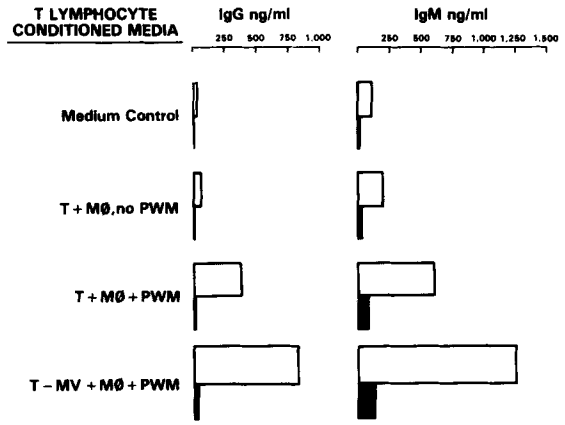

FIGURE 1. Measles virus suppresses B lymphocytes, not T lymphocytes and monocytes, in PWM-driven Ig synthesis. B lymphocytes were cultured in TCM from uninfected (T + Mϕ) or measles virus–infected (T − MV + Mϕ) T lymphocyte monocyte cultures as described in Materials and Methods. Open bars, Ig secretion from mock-infected B cells; closed bars, Ig secretion from virus-infected B cells.

than TCM from mock-infected T lymphocytes for mock-infected B cells. As seen in Fig. 1, the enhancement ranged from two- to threefold. Whether infected B lymphocytes were cultured in TCM from mock-infected or infected T cells, Ig secretion did not increase significantly above a minimum negative control level. Most importantly, Ig secretion from infected B cells was always suppressed compared with mock infected B cells. Suppression required infectious virus, i.e., UV-inactivated virus did not suppress Ig secretion.

The B lymphocyte population used in these experiments contained sheep erythrocyte receptor negative cells of the NK cell phenotype. NK cells can be activated in PWM cultures to suppress Ig secretion (11). Although measles virus infection of NK cells has been shown to abrogate their lytic function (7), it was necessary to rule out a potential suppressor function by these cells. NK cells were excluded by depleting Leu-7$^+$ and Leu-11$^+$ cells from the B lymphocyte population by FACS (Fig. 2*A*). The sorted, uninfected B cells (<2% NK contamination by immunofluorescence and 95% reduction of natural cytotoxicity) secreted IgG and IgM when cultured in TCM. Virus-infected B cells were suppressed. Adding NK cells back at 50 or 100% of the number of B cells in culture, which exceeded the percentage of NK cells in the unsorted population, caused a minor reduction in the response of mock-infected cultures and no change in the suppression of infected cultures (Fig. 2*B*). Thus, NK cells do not play a role in measles virus suppression of B cell function.

Does virus induced suppression of B cell function result from lytic infection? B cells, mock- or virus-infected, were cultured in TCM, as described above. In three experiments, at successive times from initiation of culture the numbers of viable cells were similar in infected and in mock-infected cultures at days 1, 3, 5, and 7 of incubation. In parallel cultures, cell proliferation was assayed. On days 3 or 5 of culture, [^{3}H]thymidine incorporation by mock-infected B cells increased fivefold above proliferation on day 1, but there was no increase above a low background level by virus-infected B cells ([^{3}H]thymidine cpm, mean ± SD:

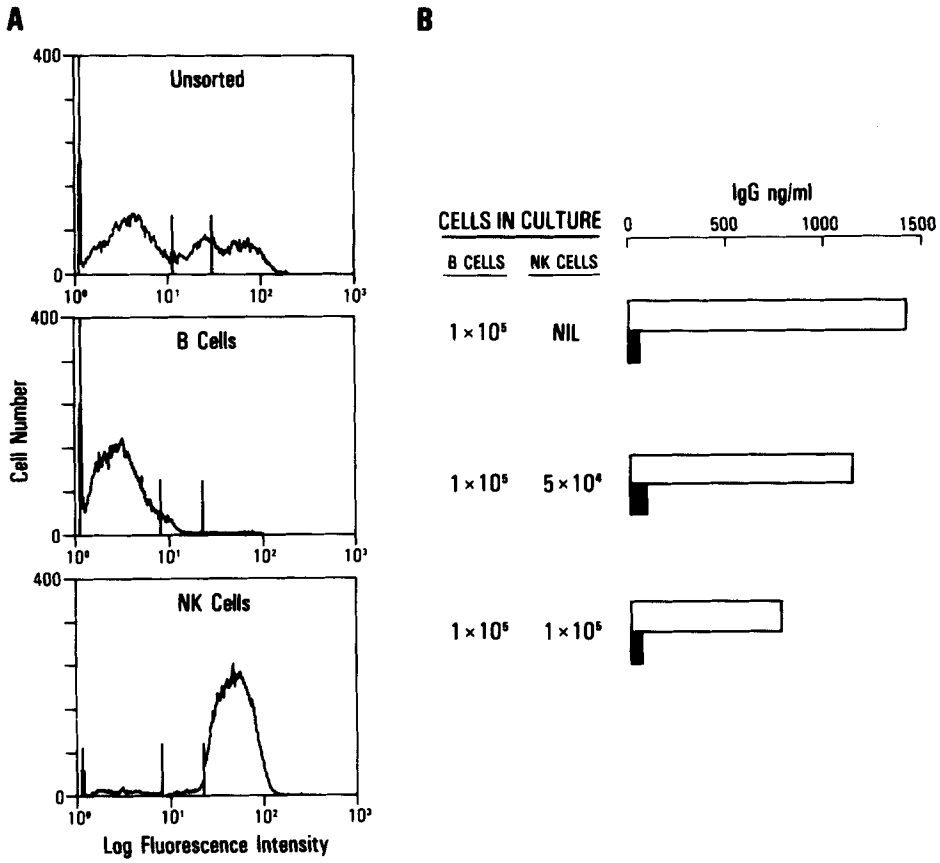

FIGURE 2. (*A*) Flow microfluoremetry analysis of the unsorted B cell population (top), negatively sorted B cells (middle) and positively-sorted NK cells (bottom), using mAbs anti-Leu-7 and anti-Leu-11 (Materials and Methods). Vertical line marks on the abscissae indicate the channel gates selected for sorting. (*B*) B lymphocytes were cultured in TCM alone or with NK cells added back. Open bars, Ig secretion from mock-infected B cells; closed bars, Ig secretion from virus-infected B cells.

mock-infected, 2,511 ± 271 on day 1, 12,746 ± 1,698 on day 3, and 12,709 ± 3,208 on day 5; measles-infected, 852 ± 358 on day 1, 1,219 ± 265 on day 3, and 877 ± 257 on day 5). Viral antigens could be detected in 85–95% of infected B lymphocytes by surface immunofluorescence on day 3 or day 7 of culture, and infectious virus could be recovered from cultures after 24 h of incubation. Similarly, measles virus was observed in B lymphocytes by electron microscopy (Fig. 3).

The conclusions drawn from these experiments are: (*a*) measles virus suppression of PWM-driven Ig secretion is mediated by infection of B lymphocytes, not T lymphocytes, monocytes, or NK cells; (*b*) suppression is not caused by lytic infection; and (*c*) infected T cells and monocytes have no defect in the secretion of B cell growth and differentiation factors when stimulated by PWM. Indeed, production of these factors may be enhanced during the virus infection. Two predictions seem reasonable. Firstly, the observation that virus added to PWM

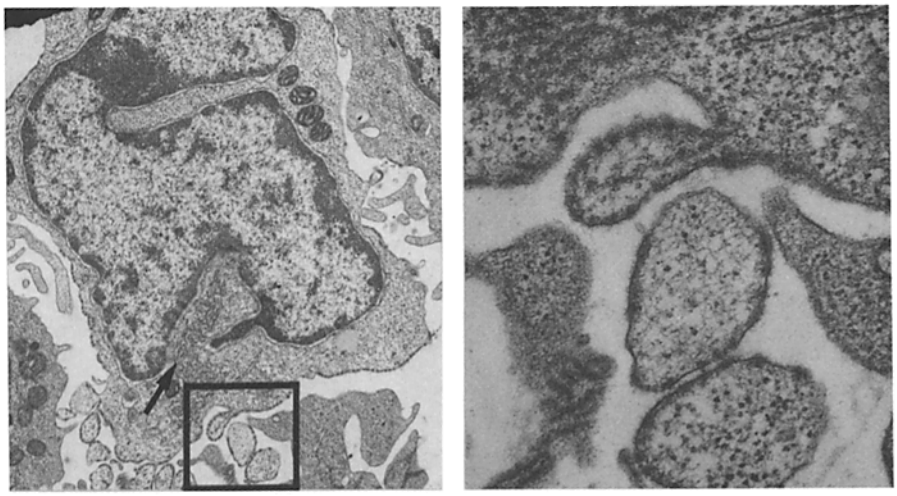

FIGURE 3. Two electron micrographs of measles virus infection of B lymphocytes. Infected cells were cultured with PWM and TCM. Left, nucleocapsid particles can be seen in the cytoplasm (arrow), × 6,000. Box, enlarged area that shows a virion budding from the plasma membrane, × 20,000.

cultures after day 3 does not suppress Ig secretion (7) suggests that measles virus infection arrests early events in B lymphocyte differentiation. This may explain the clinical observation of the inability of patients to make antibodies to a variety of challenge antigens early during infection (4). Secondly, the apparent enhanced synthesis of B cell growth and differentiation factors during measles virus infection may, in part, play a role in the polyclonal activation of already stimulated B lymphocytes in vivo (12) and the production of autoantibodies (13).

In a contemporary model of human B lymphocyte function in vitro, discrete stages of activation, proliferation, and differentiation have been defined (reviewed in reference 14). Small, resting B cells, when triggered by agents that bind surface Ig, become activated and express receptors for B cell growth factors. In the presence of these factors, B cells are driven to proliferate and express receptors for differentiation factors. In turn, these factors drive B cells to differentiate into Ig-secreting cells. In the context of this model, measles virus suppression of B cell function would likely occur during activation or early proliferation stages. Preliminary data suggest that the stage of B lymphocyte differentiation blocked by measles virus infection is early proliferation (McChesney, M., and J. Kehrl, unpublished observations).

Summary

Measles virus infection is associated with suppression of immune functions both in vivo and in vitro. The virus infects T lymphocytes, B lymphocytes, and monocytes, but does not produce cytolysis. One consequence of infection in vitro is the failure of T and B lymphocyte mixtures to cooperate in secreting Ig in a PWM-driven system. Here we report that this defect in Ig secretion resides in the infected B lymphocyte, but not in the T lymphocyte or monocyte. Further, NK cells are not involved, since neither their depletion nor reconstitution abrogates suppression of B cell function. Proliferation of B cells in the early

culture period is suppressed, suggesting that measles virus suppresses B cell development at the activation or proliferation stages, but does not affect terminal differentiation into Ig secreting cells.

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