

VIRUSES DISRUPT FUNCTIONS OF HUMAN LYMPHOCYTES

Effects of Measles Virus and Influenza Virus on Lymphocyte-mediated Killing and Antibody Production

BY PAOLO CASALI, GEORGE P. A. RICE, AND
MICHAEL B. A. OLDSTONE

*From the Department of Immunology, Scripps Clinic and Research Foundation,
La Jolla, California 92037*

The ability of a virus to alter an expected immune response first received recognition over three-quarters of a century ago. At that time, Clements von Pirquet (1) observed that children who were tuberculin positive before contracting acute measles virus infection failed to mount specific skin responses to tuberculin during that virus infection. Later reports confirmed von Pirquet's observation and extended his findings to other infectious agents (2–10). Further, during acute measles virus infection, humans may not make antibodies to tetanus toxoid or H and O antigens of *Salmonella typhi* (11). Lymphocytes harvested from individuals undergoing acute measles virus infection respond poorly, in vitro, to a variety of mitogenic (12–15) or antigenic stimuli (11, 14, 15) and are deficient in making chemotactic factors (15, 16). During measles virus infection, pulmonary tuberculosis (17, 18) worsens and lipoid nephrosis, a disease that frequently responds to immunosuppressive therapy, improves (19, 20).

Contemporary immunology is largely concerned with identification of immunocompetent cells necessary for a wide variety of immune responses and of factors that modulate these responses. Viruses are important modulators for their ability to infect immunocompetent cells and alter immune responses (21–24). For example, measles virus antigens are expressed in lymphocytes harvested from patients during acute infection (14, 25), and infectious virus can be recovered from lymphocytes during the acute and persistent infections (14, 26–29). In vitro, measles virus can replicate in monocytes, B lymphocytes, T lymphocytes bearing Fc receptors for both IgG and IgM (30–32), and T lymphocytes bearing OKT4 and OKT8 markers (33, 34).

Because immunologic dysfunctions (autoimmunity, immunosuppression) may result from either an active or a "silent" virus infection, we have undertaken to study interactions between viruses and human lymphocytes. The questions addressed in this report are threefold. First, can measles virus and, for comparison, influenza virus directly alter the performance of lymphocytes as natural killer

This is Publication No. 2874 from the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA. This work was supported by USPHS grants AI-07007, AI-09484, NS-12428, Weingart Foundation Funds, NATO Grant 069.81 and General Clinical Research Center support RR00833. Dr. P. Casali was a recipient of a Clinical Investigation Award from the Medical Group of Scripps Clinic and Research Foundation and Dr. G. P. A. Rice of a Centennial Fellowship from the Medical Research Council of Canada.

(NK)¹ cells or as effector (K) cells of antibody-dependent cell-mediated cytotoxicity (ADCC)? Second, can these viruses alter the synthesis of immunoglobulins (Ig)? Third, does abrogation of lymphocyte function by viruses occur without virus-induced cytotoxic effects? Here we present evidence that measles virus and influenza virus significantly alter selected lymphocyte functions in vitro. This occurs without morphologic injury to the infected lymphocytes.

Materials and Methods

Virus Source and Virus Growth. The Edmonston strain of measles virus obtained from the American Type Culture Collection, Rockville, MD was plaque purified and handled as previously described (35, 36). After growth on Vero cells the viral titer was $2-7 \times 10^7$ plaque-forming units (pfu)/ml. Inactivation involving exposure of measles virus to ultraviolet light ($1,200 \text{ ergs/cm}^2/\text{s}$) for 4 min reduced the titer to 4×10^5 pfu/ml. Influenza virus strain A/WSN/33 (H1N1) was a gift from Dr. Richard W. Compans (University of Alabama, Birmingham, AL) and had a titer of 1×10^8 pfu/ml.

Immunochemical Reagents. Antibodies to measles virus glycoproteins were obtained from the serum of a patient with subacute sclerosing panencephalitis (SSPE). In this serum, measles virus-specific antibody accounted for 12% of the total Ig (35) and the F(ab')₂ fragments of SSPE IgG were prepared by digestion with pepsin (35). Polyclonal specific antibody to influenza virus and to P-815 cells was made in rabbits. For studies of measles virus-infected cells, SSPE IgG was conjugated directly with fluorescein isothiocyanate; influenza virus-infected cells were examined with rabbit antibody to influenza virus and fluorochrome-conjugated goat antibody to rabbit IgG. In several studies monoclonal antibodies to measles virus hemagglutinin, measles virus nucleoprotein, WSN hemagglutinin, and nucleoprotein, HLA-DR, human T cells, and NK cells were used. The preparation of these reagents, their specificities, and their use in immunofluorescence assays have been reported (37).

Peripheral Blood Lymphocytes (PBL) and Lymphocyte Subsets. Blood obtained from healthy males and females, ages 23–50 years, either seropositive or seronegative for measles virus or influenza virus, was placed on Ficoll-Hypaque (Pharmacia, Piscataway, NJ) gradients (36, 38). Mononuclear cells were freed from adherent cells by culturing twice for 60 min at 37°C in a plastic 175-cm² flask, resulting in a preparation with <2% macrophages, as tested by dye exclusion or esterase analysis (36). This mononuclear population is referred to here as PBL. NK or killer (K) cells were negatively selected by lysis of PBL with monoclonal antibody OKT3 (Ortho Pharmaceuticals, Raritan, NJ) and antibody to human HLA-DR (Becton Dickinson, Sunnyvale, CA) and complement. Briefly, 1.5×10^8 PBL suspended in 15 ml of 2% RPMI containing 2% fetal bovine serum were incubated with 10 µg of antibody to OKT3 and 25 µg of antibody to human HLA-DR for 30 min at room temperature and 30 min at 4°C. The mixture was incubated for 60 min at 37°C with rabbit complement. Viable cells were recovered on Ficoll-Hypaque gradients. To separate B and T lymphocyte subsets, PBL were fractionated into erythrocyte rosetting and nonrosetting cells by using two Ficoll-Hypaque gradients and neuraminidase-treated sheep erythrocytes as described (32, 36).

Incubation of PBL with Viruses. PBL ($10^7/\text{ml}$) were infected at a MOI of 2 or 3 for 1 h at 37°C. Control PBL received virus-free culture fluid or fresh medium. Cells were diluted, washed extensively in warm (37°C) media, and diluted to $1-1.5 \times 10^6/\text{ml}$ in T-75 flasks and incubated at 37°C, 5% CO₂. After 24, 48, 72, and 144 h in culture, samples of cells were analyzed for viability by trypan blue dye exclusion and for expression of viral antigens by immunofluorescence. In some experiments, surface phenotypic analysis of

¹ Abbreviations used in this paper: ADCC, antibody-dependent cell-mediated cytotoxicity; ELISA, enzyme-linked immunosorbent assay; IFN, interferon; Ig, immunoglobulin; K cell, killer cells; MOI, multiplicity of infection; NK, natural killer; PBL, peripheral blood lymphocytes; PFU, plaque-forming unit; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SSPE, subacute sclerosing panencephalitis.

cultured PBL was performed with monoclonal antibodies to human lymphocyte antigens, and automated cytofluorometry. Supernatants were tested for infectivity (PFU), interferon (IFN) levels, or activity and Ig production (36, 39–41).

Target Cells. Cell lines used as targets were adult human skin fibroblasts, human newborn foreskin fibroblasts, K-562 cells, rhabdomyosarcoma cells, and murine P-815 cells. Skin fibroblasts were established in culture as previously described (36) and HLA type was obtained by the Clinical Immunology Laboratory of Scripps Clinic and Research Foundation.

Cytotoxicity Assay. Target cells were placed in 96-well plates after labeling (10^4 target cells/well) with ^{51}Cr as described (36). At least two effector-to-target cells ratios were used and specific lysis calculated after 8 h incubation by the formula:

$$\% \text{ specific release} = \frac{Sam - Spo}{Max - Spo} \times 100,$$

in which *Spo* is the ^{51}Cr released from targets in the presence of medium alone; *Max* is the ^{51}Cr released by the targets after lysis with the detergent NP-40, and *Sam* is the amount of ^{51}Cr released in experimental samples to be tested. Tests were run in triplicate, and spontaneous release did not exceed 15%.

IFN Assay. Interferon was obtained, used, and titrated as described (36, 40). One interferon unit described in our experiments was equivalent to 1 U of NIH reference interferon (G-023-901-5271).

Proliferation of PBL Cultured with Virus. 3×10^5 PBL incubated with measles virus or influenza virus in a final volume of 0.3 ml were placed in a 96-well plate (Costar, Cambridge, MA) with phytohemagglutinin (PHA), (1 $\mu\text{g}/\text{ml}$) and radiolabeled thymidine or deoxyuridine added 24 h later. DNA and RNA synthesis was evaluated by measuring the amount of TCA-precipitated radioactive counts incorporated within 24 h.

Ig Synthesis by Lymphocytes. 1×10^5 B lymphocytes and 2×10^5 or 3×10^5 T lymphocytes were plated in 96-well plates. Either measles virus or influenza virus was added at a MOI of 2–3. Pokeweed mitogen (PWM, Gibco) was added (5 $\mu\text{g}/\text{ml}$) and cells cultured for 7 d at 37°C in 5% CO_2 . Supernatant fluids were harvested at the seventh day of culture and tested for amounts of IgM and IgG with a modified enzyme-linked immunosorbent assay (ELISA) incorporating affinity-purified sheep antibodies to human IgM or IgG (40, 41). Controls consisted of uninfected B and T lymphocytes in similar ratios or uninfected B lymphocytes maintained under identical culture condition with PWM.

Results

NK Cell Activity Is Abrogated by Measles Virus Infection of Effector Lymphocytes. Equal numbers of PBL from seven human donors were infected with measles virus at an MOI of 3 or were mock infected. Both samples from each donor were then cultured under similar conditions and assayed for their ability to kill the NK cell-sensitive targets K-562, rhabdomyosarcoma, and adult skin fibroblast cells, at 24, 48, and 72 h after initiation of infection. During the first 6, 8, 12, or 24 h, NK lytic activity proceeded as expected (data not shown) (36). For example, PBL from donor 1, infected with measles virus, were roughly equivalent to uninfected PBL from the same donor in lysing K-562 cells (measles virus-infected PBL: 61% specific ^{51}Cr release, uninfected PBL: 73% specific ^{51}Cr release) (Fig. 1). However, after 48 and 72 h of measles virus infection, killing by infected PBL of K-562 cells decreased to 22% and 3% specific ^{51}Cr release, respectively. In contrast, uninfected PBL showed no defect in killing at 48 and 72 h, releasing 77% and 78% ^{51}Cr . Similar results were obtained at effector-to-target cell ratios in the range of 50:1, 20:1, 10:1, 5:1 on all the target cell lines with seven different donors. The results for three donors are illustrated

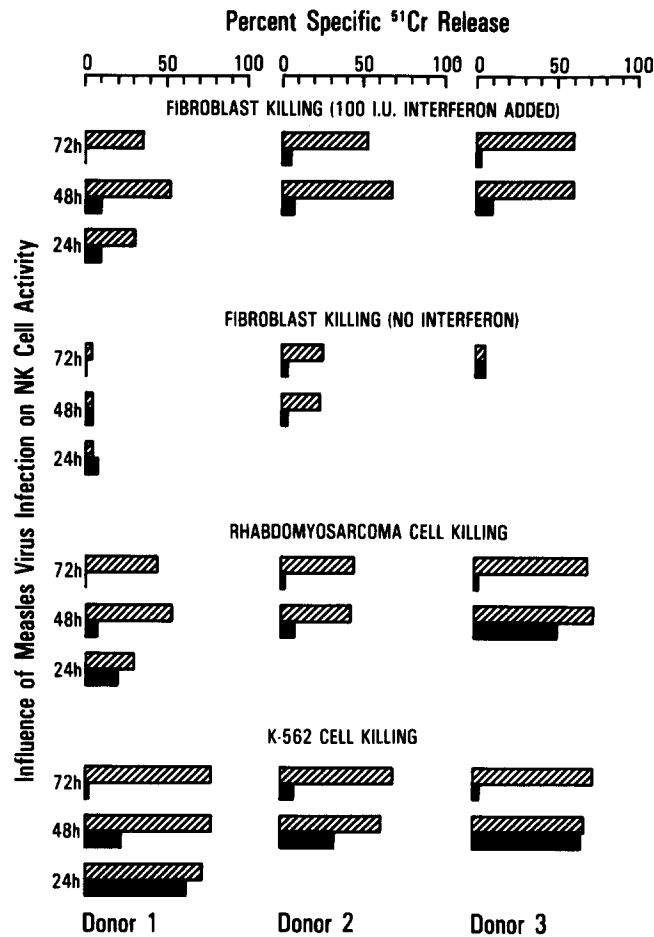


FIGURE 1. Measles virus inhibits NK cell activity. Measles virus was used to infect (MOI 3) PBL from three donors. At 24, 48, or 72 h after infection, PBL were added to ⁵¹Cr-labeled K-562 cells, rhabdomyosarcoma cells, or skin fibroblasts. Specific ⁵¹Cr release was monitored and compared to that from uninfected PBL. Skin fibroblasts were not HLA-A or -B matched with donor PBL and were cultured in the absence or presence of 100 IU of α interferon. Effector/target ratio of 50:1 was used. Concurrently run assay at ratio of 10:1 gave corresponding results (data not shown). All samples were run in triplicate and the spontaneous ⁵¹Cr release did not exceed 15%. (■) Measles virus infected; (▨) uninfected lymphocytes.

in Fig. 1. Equivalent lysis occurred with HLA unmatched and matched fibroblast targets. In data from all donors, PBL infected with measles virus for 72 h and incubated with all three target cells yielded an average value of 92% (range 75% to 100%) inhibition of the expected NK cell lytic activity experimentally obtained by NK cell activity of uninfected control PBL. The viability of effector PBL incubated with measles virus for 24–72 h exceeded, on the average, 90% (average 77–99%) and was not different from that of uninfected PBL. Further, the proportion of PBL identified with the NK-specific monoclonal antibody, B73.1, did not change during culture over a 96-h period (data not shown). Thus, measles virus infection abrogated the expected NK activity of PBL, without specific lysis

of the NK cell subset. These findings were further documented in experiments shown in Table I (see below).

Next, to determine whether interferon added to target cells enhanced their killing by measles virus-infected PBL, as is characteristic for NK cells, we added 100 IU of human alpha IFN to cultured human adult skin fibroblasts. Such cells are relatively poor NK activity inducers unless IFN is added, and they do not spontaneously make IFN. As seen in Fig. 1, despite the addition and maintenance of 100 IU of IFN per ml during the cytotoxic assay, PBL infected with measles virus were still unable to kill these fibroblast target cells, whereas uninfected PBL showed enhanced NK cell activity.

Human NK cell activity resides primarily in a non-B, non-T lymphocyte subset that bears Fc receptors (reviewed in reference 42). We obtained a NK-enriched population of cells by depleting PBL of T and B cells using antibody to OKT3, antibody to HLA-DR, and complement. In separate experiments with PBL from three individual donors, 10–15% of starting cells remained alive after this procedure. <5% of these cells bound either OKT3 or HLA-DR antibody but >90% bound monoclonal antibody B73.1, a marker for NK cells (42, 43), indicating a population of highly enriched PBL with potential NK and K cell activity. This subset of PBL was divided so that half could be infected with measles virus at a MOI of 3 and the other half mock infected (uninfected control), but otherwise handled and manipulated identically. As seen in Table I, the enriched population of NK cells infected with measles virus killed K-562 or

TABLE I
*Measles Virus Abrogates the Lytic Activity of an Enriched Subset of NK Cells**

PBL from donor	Measles virus	% Viable cells	NK cell targets		
			K-562	Fibroblasts: [‡] Nil	IFN
1 (48 h)	No	90	ND [†]	17 [†]	46
	Yes	84	ND	22	21
2 (48 h)	No	89	53	4	35
	Yes	89	9	5	3
3 (72 h)	No	85	68	9	55
	Yes	76	13	9	10

* PBL were depleted of adherent cells and of OKT3- and HLA-DR-bearing cells by using monoclonal antibodies and complement. These purified NK cells were <5% OKT3⁺, HLA-DR⁺, and >90% B73.1⁺ cells. NK cells from three donors were infected (MOI 3) with measles virus or mock infected and handled identically. Effector-to-target ratio was 5:1. Lymphocyte viability was measured by trypan blue dye exclusion using at least 200 cells. Length of time of incubation of lymphocytes with measles virus is in parentheses.

[‡] Fibroblasts (adult skin, non HLA matched) were either incubated with 100 IU/ml of human α -IFN present throughout the assay or left untreated (nil).

[†] Not done.

[‡] Percent specific ⁵¹Cr release. Data represent the mean value from triplicate samples. Spontaneous release did not exceed 15%.

adult fibroblast targets less efficiently than the mock-infected cells. Yet, their viability was similar to mock-infected cells or to unsegregated lymphocytes, indicating that preferential lysis of a lymphocyte subset by measles virus did not occur. >85% of these purified NK cells, initially incubated with virus, expressed measles virus antigens after the addition of PHA, indicating that the majority of the effector cells were infected. At rest, <5% of such cells expressed virus antigens.

K lymphocytes mediate ADCC and bear many of the same markers as NK cells (42). Therefore, we evaluated the ability of measles virus-infected PBL and the infected non-B non-T enriched subset to participate in an ADCC assay. For these experiments, murine P-815 cells were radiolabeled with ^{51}Cr and optimally sensitized with antibody to P-815. Such radiolabeled sensitized cells were incubated with either lymphocytes infected for 24, 48, or 72 h with measles virus or incubated with matched cultured uninfected lymphocytes. All seven experiments performed with PBL from five donors, (data not shown) had identical results. The representative example is donor 1 (in Fig. 1) whose PBL used at an effector-to-target ratio of 10:1 after infection with measles virus for 24, 48, or 72 h caused the release of 57, 61, and 60% specific ^{51}Cr from sensitized P-815 cells; at similar times matched uninfected PBL released 65, 51, and 62% ^{51}Cr . Similarly, after 72 h of measles virus infection, lymphocytes from donor 2 released 60% ^{51}Cr from sensitized P-815 targets as compared to 62% by matched uninfected lymphocytes. Thus, with the system used, measles virus infection of lymphocytes does not abrogate their ability to perform in ADCC. That ADCC was spared in infected PBL that have lost their NK function further supports the contention that a selective cytotoxic effect of measles virus on a selected NK subset did not occur because these functions are mediated by the same cell (42). Thus our data support the concept of a "silent infection," and do not support the thesis of selective cytolysis.

Antibody to Measles Virus Prevents the Inhibition of NK Cell Activity Associated with Measles Virus Infection of Effector Lymphocytes. Antibody able to neutralize measles virus (1 ml neutralized 1×10^7 pfu) and immunoglobulin not containing antibody to measles virus were processed to make F(ab')_2 fragments. Measles virus was incubated with F(ab')_2 from either preparation for 1 h at 37°C and then added to PBL at a MOI of 3. 72 h later, these cells were combined with K-562, skin fibroblasts, or P-815 targets previously labeled with ^{51}Cr . As seen in Fig. 2, pretreatment of measles virus with F(ab')_2 antibody to measles virus blocked the ability of measles virus to turn off NK cell lysis (K-562, fibroblast targets) but had no effect on ADCC (P-815 targets coated with antibody to P-815 cells).

Abrogation of NK Cell Activity by Measles Virus Infection Is Not from Soluble Factors Released by Infected Lymphocytes. To determine whether virion-free soluble factors released from virus-infected PBL played a role in inhibiting NK lymphocyte killing, supernatant fluids were harvested at 24, 48, and 72 h from measles virus-infected PBL, centrifuged at $100,000 \text{ g}$ for 1 h at 4°C , and filtered through a 0.4- μm filter to remove virions. The eluates were incubated with 1×10^6 PBL for 24 h and then mixed with ^{51}Cr -labeled target cells. These culture fluids from PBL of six donors all failed to inhibit NK cell-mediated lysis, as the representative sample in Fig. 3 shows. Furthermore, preincubation of PBL with exogenous

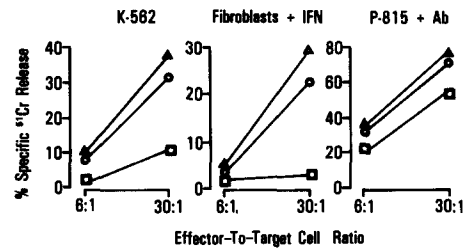


FIGURE 2. F(ab')₂ fragments to measles virus reverse the abrogation of NK cell activity measles virus. PBL from one donor were either not incubated with measles virus (○) or were incubated at a MOI of 3 with measles virus, preparation previously treated with nonimmune F(ab')₂ fragments (□) or with F(ab')₂ fragments to measles virus (Δ). After 72 h in culture, PBL were assayed for their ability to lyse ⁵¹Cr labeled K-562 cells (left panel), fibroblasts incubated with 100 IU of α -interferon (IFN) (central panel) or P-815 cells coated with antibody to P-815 cell surface determinants (right panel). Results represent the mean of triplicate samples. Results were similar with PBL from two other donors.

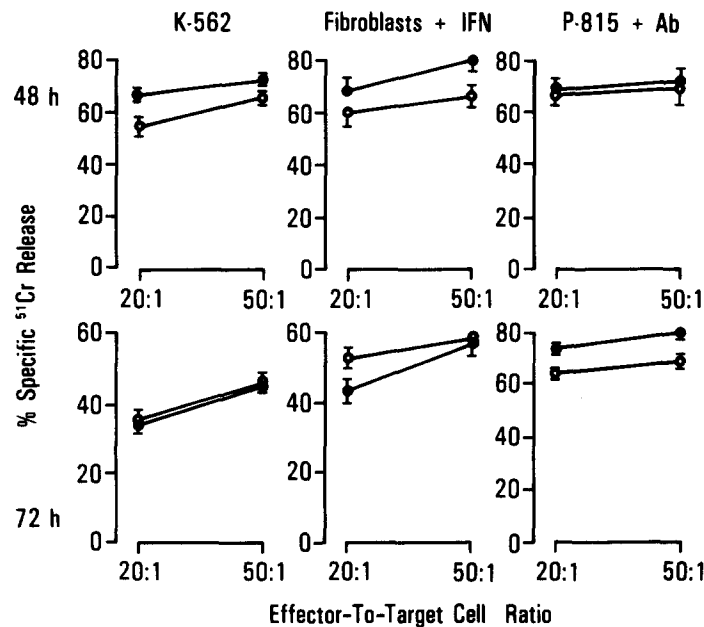


FIGURE 3. Soluble factors released from measles virus infected PBL do not inhibit NK cell activity. PBL were cultured for 48 or 72 h in culture media harvested from uninfected lymphocytes (○) or media harvested from PBL 72 h after infection with measles virus (●). Fluids were cleared of particulate material (virus) by centrifugation and filtration (see Materials and Methods section). Results represent mean value \pm 1 SE from 6 donors in triplicate.

alpha IFN, in concentrations similar to that measured in the infected cultures (100–200 IU), did not inhibit NK cell lysis (data not shown).

NK Cell Activity Is Not Abrogated by Influenza Virus Infection of Effector Lymphocytes. Influenza virus infection, like measles virus infection, can deter its host's immune responsiveness to a variety of non-viral antigens (9, 10). In our studies (see below), influenza virus replicated in human PBL without impairing their viability. For this reason, we tested whether the expected cytotoxic function of

NK cells and K cells was impaired by infection with influenza virus. A/WSN/33 strain of influenza virus was used at a MOI of 3 to infect PBL. Infected and mock-infected PBL were cultured, handled, and used as described for measles virus-infected PBL. PBL from four donors exposed to influenza virus for 72 h had a viability of 90% or greater. By immunofluorescence assay, >70% of these infected cells expressed viral antigens in their cytoplasm or on their surfaces, although infectious virus was not detected in culture fluids. However, unlike the interference by measles virus, influenza virus infection did not deter PBL harvested at 24, 48, or 72 h from generating NK cell and ADCC activity. Results for PBL for all four donors are represented by the data from one donor given in Table II. These PBL killed K-562 targets and skin fibroblasts with or without IFN present and also killed P-815 cells precoated with antibody to P-815 surface determinants to the same degree as did uninfected PBL.

TABLE II
*Influenza Virus Does Not Abrogate the Lytic Activity of NK Cells**

Time of virus infection	Influenza virus infection	% specific ⁵¹ Cr release from:									
		NK cell activity						ADCC			
		K-562		fibroblasts [‡]				P-815 [§]			
		50:1	15:1	nil		with IFN		nil		with Ab	
		50:1	15:1	50:1	15:1	50:1	15:1	50:1	15:1	50:1	15:1
48 h	No	53 ± 1	25 ± 1	8 ± 3	6 ± 1	54 ± 1	32 ± 1	3 ± 1	4 ± 1	69 ± 5	60 ± 1
	Yes	63 ± 2	34 ± 4	36 ± 5	23 ± 1	45 ± 7	26 ± 2	13 ± 2	10 ± 1	72 ± 3	52 ± 10
72 h	No	45 ± 7	27 ± 5	17 ± 2	11 ± 2	65 ± 1	22 ± 3	8 ± 1	12 ± 2	87 ± 2	71 ± 2
	Yes	48 ± 2	31 ± 6	38 ± 5	17 ± 4	66 ± 2	21 ± 2	12 ± 3	11 ± 1	97 ± 3	70 ± 3

* PBL were infected (MOI 3) with influenza virus or mock infected and handled identically. At the time of the assay the viability of cultured infected or uninfected PBL was equivalent and exceeded 90%, as measured by trypan blue dye exclusion. Data given are from one of four donors, but all showed equivalent results.

[‡] Fibroblasts (adult skin, non HLA matched) were not treated (nil) or treated with IFN (100 IU/ml of human α -IFN present throughout the assay).

[§] P-815 cells were either not treated (nil) or were coated with antibody to P-815 cell surface determinants.

^{||} Effector-to-target cell ratio.

[†] Percent specific ⁵¹Cr release. Data represents the mean value \pm 1 SE. Samples run in triplicate and spontaneous release did not exceed 15%.

TABLE III
Measles Virus or Influenza Virus Infection Abrogates the Synthesis of Ig in Mixtures of B and T Lymphocytes

Donor	Lymphocytes* infected with:									
	Nil		Measles virus [‡]				Influenza virus [‡]			
	IgM	IgG	Virus		UV-virion		Virus		UV-virion	
			IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
5	93 [§]	140	6	4	74	103	6	4	97	100
10	278	303	6	4	320	82	8	4	700	113
19	313	167	20	12	253	300	30	15	700	123
20	466	533	28	4	ND	200	30	4	ND	16

* 1×10^5 B lymphocytes cultured with 2×10^5 T lymphocytes in the presence of PWM (5 μ g/ml) as described in Materials and Methods.

[‡] Infectious virus or virus inactivated by UV light before its incubation with lymphocytes (see Materials and Methods for results).

[§] ng of Ig produced by 1×10^5 B lymphocytes after 6 d of culture.

^{||} Not done.

Ig Synthesis Is Abrogated by Either Measles Virus or Influenza Virus Infection of Lymphocytes. We next investigated the ability of PBL, containing B and T helper lymphocytes to synthesize IgG and IgM in the presence of either measles virus or influenza virus infection. In these studies B and T lymphocytes were mixed in a ratio of 1:2, or 1:3, infected at a MOI of 2 or 3 with either measles or influenza virus and cultured in the presence of PWM for 6–7 d. At this time cell viability was equivalent in uninfected and infected cultures and exceeded 80%. Additionally, >80% of the cells infected with measles virus or influenza virus expressed viral antigen. These infections significantly dampened the synthesis of both IgM and IgG as indicated in Table III. Two approaches indicated that abrogation of Ig synthesis was dependent on replicating virus: first, UV-inactivated virus failed to abrogate Ig synthesis and second, pretreatment of infectious measles virus with $F(ab')_2$ fragments of antibody to measles virus negated inhibition of Ig synthesis. Table III also shows that PBL from donors 10 and 19 increased Ig synthesis when cultured with UV-inactivated virus. These results, observed on several occasions, remained unexplained but did not relate to the titers of influenza or measles virus antibody carried by these donors.

Next we determined the time course for the inhibition of Ig synthesis by infectious virus. Fig. 4 demonstrates that adding measles virus at the start of B and T lymphocyte culturing with PWM up to 36 h thereafter was sufficient to markedly reduce Ig synthesis. Even after 72 h, the addition of virus to these cultures inhibited PWM-driven Ig synthesis by >70%. However, when measles virus was added 144 or 168 h after initiating B and T lymphocyte cultures, no inhibition of Ig synthesis occurred. Apparently, measles virus acts on lymphocytes at an early stage but cannot block fully differentiated cells from synthesizing Ig.

Infection of PBL by Either Measles Virus or Influenza Virus. PBL infected at a MOI of 3 with either measles virus or influenza virus with or without the mitogen PHA, were assayed for expression of viral antigens and ability to incorporate

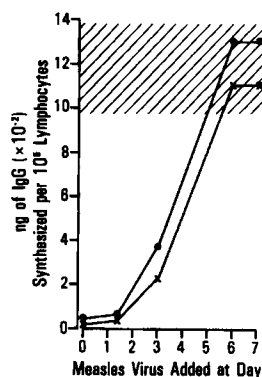


FIGURE 4. Measles virus infection of PBL abrogates their synthesis of Ig. 1×10^5 B lymphocytes were mixed with 2×10^5 T lymphocytes in the presence of PWM ($5 \mu\text{g/ml}$) and placed in culture. Measles virus (MOI of 3) was added on different days of culture, after which the amount of IgG synthesized was determined daily for 7 d after the initiation of PWM driven lymphocyte response. Data shows lymphocytes from two donors (●, x). Diagonal lines (////) indicate the variance of 2 SE from the mean value of IgG synthesized by uninfected lymphocytes from these two donors.

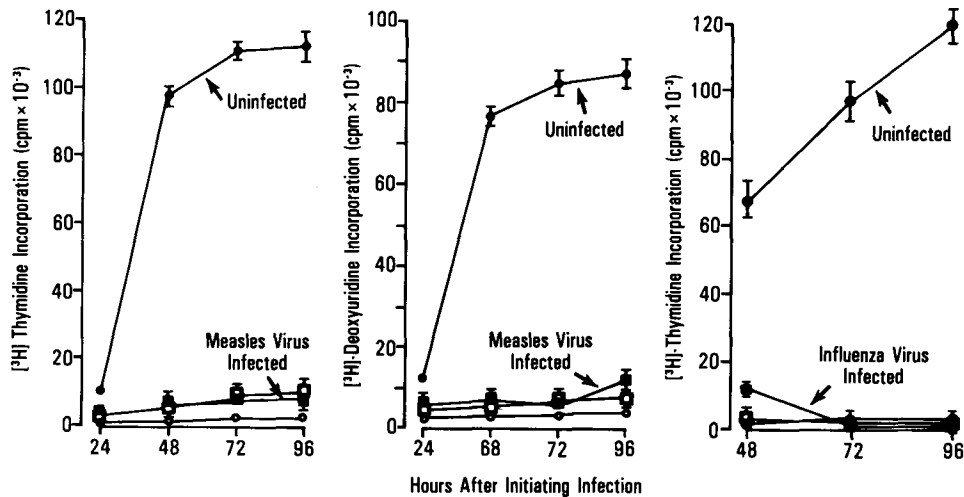


FIGURE 5. Lymphocytes infected with measles virus or with influenza virus do not proliferate in response to PHA. PBL were incubated with either virus at a MOI of 3 (\square), or were mock infected (\circ). Infected and uninfected lymphocytes were then cultured in the presence (filled symbols) or absence (open symbols) of PHA. Results represent the mean \pm 1 SE of triplicate cultures from lymphocytes obtained from one donor. Similar results occurred with lymphocytes from four additional donors.

[^3H]thymidine or [^3H]deoxyuridine into TCA-precipitable material. Resting PBL (no PHA) either uninfected or infected with measles virus or influenza virus, incorporated little [^3H]thymidine or [^3H]deoxyuridine during the 96-h observation period. On the average, <8% of these measles virus-infected PBL (range 3% to 12%) expressed viral antigens either on their surfaces or in their cytoplasm. Similarly, little infectious measles virus (<10² PFU/ml) was released into culture fluids. In contrast, >70% of resting, influenza virus (no PHA)-infected PBL expressed viral antigens both in the cytoplasm and on the surface by the third day of culture. Infectious virus was not detected in culture fluids. Both measles virus or influenza virus infection blocked lymphocytes cultured with mitogen from incorporating [^3H]thymidine or [^3H]deoxyuridine into TCA-precipitable counts (Fig. 5). Influenza virus antigens on PBL did not notably increase as a result of adding PHA. In contrast, the number of PBL expressing measles virus antigens increased from <10% at the resting stage to >70% after activation with PHA, and such culture fluids contained up to 10 times the input multiplicity of virus (data not shown). Despite this increase, lymphocyte viability was not significantly jeopardized.

Discussion

Measles virus can abort several important functions of lymphocytes including NK cell killing and Ig synthesis, but these malfunctioning lymphocytes show no sign of virus-induced cytotoxic effects. Further, no measles virus gene products are evident in the vast majority of infected lymphocytes that fail to perform NK activity. Hence, the function of a differentiated cell, in this case a lymphocyte, may be turned off by a virus, yet the infected cell neither undergoes structural

abnormalities nor expresses viral antigens. Once such lymphocytes are driven to proliferate with the mitogens PHA or PWM, measles virus antigens are easily detectable in the infected cells. Considering the numerous human diseases of unknown etiology involving faulty synthesis of (a) immune regulators, i.e., acquired immune deficiency syndrome, (b) insulin or growth hormone, i.e., diabetes and disorders of growth and metabolism, or (c) neurotransmitters, i.e., Alzheimer's and Parkinson's disease, one might speculate whether these may be associated with a similar type of "silent" virus infection. Support for this hypothesis recently came from *in vivo* studies showing that persistent infection of growth hormone-producing cells in the anterior lobe of the pituitary gland resulted in clinical and biochemical disorders of growth and glucose metabolism (44). The virus produced no cytotoxic effect on growth hormone-producing cells, but altered the synthesis of growth hormone.

Abrogation of lymphocyte function by measles virus apparently depends on infecting cells before their differentiation because this virus failed to alter the function of cells already committed to that function. Hence, exposure of lymphocytes at the time of initiating measles virus infection or 3 d thereafter decreased the cells' ability to synthesize Ig (Fig. 4). In contrast, infecting lymphocytes 5–7 d after initiating B plus T lymphocyte interaction in a PWM-driven system failed to alter the expected synthesis of Ig. These results compliment studies of measles virus infection of committed cytomegalovirus (CMV) cytotoxic T lymphocytes (CTL). Such human CTL are restricted in killing of CMV-infected targets sharing HLA-A or B molecules with CTL (45). Upon addition of measles virus (MOI 2–5) these CTL continue to demonstrate lytic activity.² Our attempts to determine whether measles virus shut off Ig synthesis, by infecting a unique, rather than multiple, lymphocyte subsets, has not yielded clear results. This may not be surprising, as measles virus can infect B lymphocyte and T lymphocyte subsets (31–34), and addition of mitogens enhanced the expression and release of infectious virus (30–34, 46).

In our studies, abrogation of NK cell activity by measles virus infection of effector lymphocytes was efficient and occurred 24 h after initiating infection (Fig. 1, Table I). This diminished killing involved infectious viral particles but not soluble factors, including IFN, released from infected lymphocytes over the 72-h course of infection. Although NK cell activity was blocked during measles virus infection, the ability of infected lymphocytes to participate in ADCC was fully maintained, confirming the earlier report of Lucas et al. (46, 47). In addition to replicating in B lymphocytes, T lymphocytes bearing helper and cytotoxic/suppressor activity, and macrophages (30–34, 48), we provide evidence here that measles virus infects a non-T non-B enriched NK cell subset.

Reactivation of a prior microbial disease (17) or the initiation of a "superinfection" by another human pathogen (49), may be associated with acute measles virus infection, likely related to the effect of virus on the immune system. For example, as early as 1904 in his textbook of medicine, Osler (17) listed a major complication of acute measles virus infection as the reactivation or initiation of tuberculosis either as a bronchopneumonia or a disseminated miliary tuberculosis. von Pirquet (1) recognized the transient loss of tuberculin reactivity in

² Barysiewicz, B., P. Casali, and J. G. P. Sissons, manuscript in preparation.

children during the course of measles virus infection. This anergy started during the prodromal stage and persisted for as long as 6 wk. von Pirquet considered that tuberculosis progressed more rapidly during an intercurrent measles virus infection and provided the first conceptual framework that a virus could induce immunosuppression. Observations of tuberculin anergy and measles virus infection have been repeatedly confirmed over the last several decades (2-7, 10). Further, impaired responsiveness to antigens other than tuberculin such as *Candida*, diphtheria toxoid, and vaccinia virus has been demonstrated in subjects given live, but not killed, measles virus (50). Such depressed immune responses observed with live virus were corrected by the concurrent administration of antibody to measles virus, again indicating need for a replicating agent and the specificity of the effect. Further, measles virus infection has been associated with a decreased antibody response to poliomyelitis (51). *In toto*, these phenomena can now be explained as a result of the direct interaction of infectious measles virus with lymphocytes and the impairment of their specific functions.

Immunodepression similar to that produced by measles virus may occur with other virus infections (reviewed in reference 10). For example, Bloomfield and Mateer (9) noted changes in skin sensitivity to tuberculin during an epidemic of influenza virus infection. We found that influenza virus was permissive for resting human lymphocytes, infecting >70% of such cells after 3 d in culture. Despite the expression of viral antigens, influenza virus-infected lymphocytes maintained their structural integrity and showed viability (>90%) equivalent to uninfected cultured lymphocytes. These findings support earlier observations in which Wilson et al. (52) found lymphocytes with influenza viral antigens on their

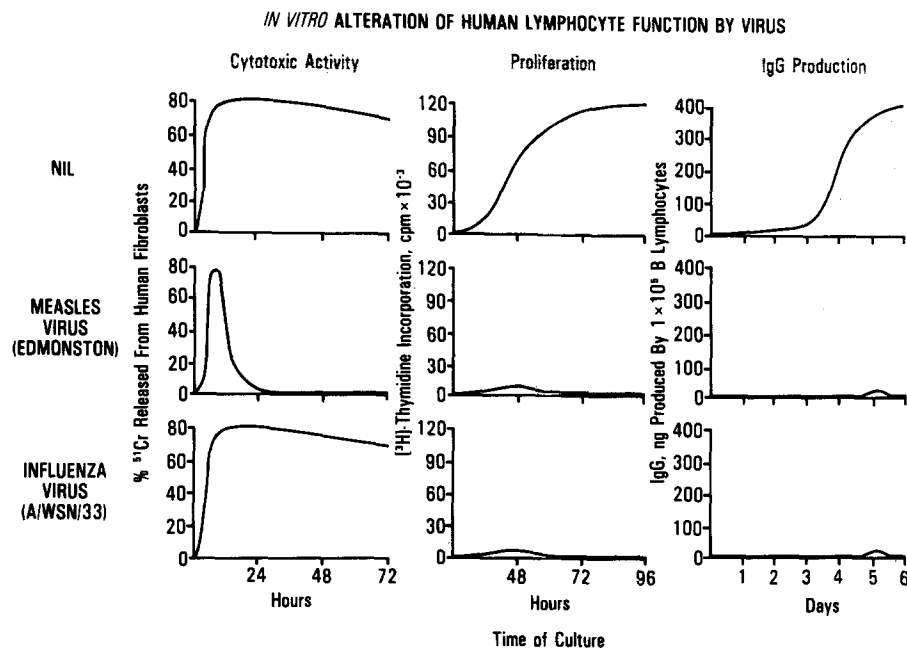


FIGURE 6. The effect of measles virus and influenza virus infection on lymphocyte functions. Nil = uninfected.

surfaces circulating in the blood of patients with acute influenza virus infection. Similar to the effect of measles virus on lymphocyte function, influenza virus-infected lymphocytes failed to proliferate in response to PHA and failed to synthesize IgG or IgM in a PWM-driven system. However, unlike measles virus-infected lymphocytes that failed to show NK cell activity, influenza virus-infected lymphocytes executed NK cell lysis as well as uninfected cultured lymphocytes. The effects of measles virus and influenza virus infection on lymphocyte functions are summarized in Fig. 6. In addition to measles and influenza viruses, herpes simplex virus has been reported to suppress Ig synthesis (53). It is of interest that herpes simplex virus suppressed Ig by acting on T helper lymphocytes, while measles virus has recently been shown to favor replication in OKT4 ("helper") rather than OKT8 ("suppressor/cytotoxic") T lymphocytes (34).

The molecular basis whereby virus infection of lymphocytes disturbs immune responsiveness is not clear. Nevertheless, both measles virus and influenza virus can infect the majority of lymphocytes without direct cytopathic effects. The in vitro model of "silent virus infection" of human lymphocytes in conjunction with the ability to clone and mark lymphocyte subsets, and the availability of genetic probes for virus and immunoglobulins makes this an interesting biologic model for future investigation.

Summary

We present experimental data that offer, in part, a better understanding of the immunosuppression that accompanies measles virus infection. We note that measles virus "silently" infects human lymphocytes and that the infection does not alter lymphocyte survival in vitro. Yet such infected lymphocytes fail to generate natural killer (NK) cell activity or synthesize immunoglobulins (Ig). Thus, the presence of virus within lymphocytes impairs their specific immune functions in the absence of cytolysis. Influenza virus also infects human lymphocytes. In contrast to measles virus infection of resting lymphocytes in which viral antigen is rarely expressed, influenza virus infection of these cells yields viral antigens expressed in the cytoplasm and on the cell surface. Influenza virus-infected lymphocytes have normal NK cell activity but fail to synthesize IgG or IgM.

We are grateful to Bice Perussia and Giorgio Trinchieri (Wistar Institute) for their generous gift of Rhabdomyosarcoma cells, K-562 cells, B73.1 monoclonal antibody, and anti-P815 serum, to Norman Kleinman (Scripps Clinic and Research Foundation) and Richard Compans (University of Alabama) for antibodies to influenza virus. We are grateful to Lisa A. Flores and Gay L. Wilkins for typing the manuscript, and to Carole J. Shoemaker for skillful technical assistance.

Received for publication 3 October 1983 and in revised form 16 January 1984.

References

1. von Pirquet, C. E. 1908. Das Verhalten der kautanen Tuberculinreaktion während der Masern. *Dtsch. Med. Wochenschr.* 34:1297.
2. Nalbant, J. 1937. The effects of contagious diseases on pulmonary tuberculosis and on the tuberculin reaction in children. *Am. Rev. Tuberc. Pulm. Dis.* 36:373.

3. Mellman, W. J., and R. Wilson. 1963. Depression of the tuberculin reaction by attenuated measles virus vaccine. *J. Lab. Clin. Med.* 61:453.
4. Starr, H., and S. Berkovich. 1964. Effects of measles, gamma-globulin-modified measles and vaccine measles on the tuberculin test. *N. Engl. J. Med.* 270:386.
5. Brody, A., and R. McAlister. 1964. Depression of tuberculin sensitivity following measles vaccination. *Am. Rev. Respir. Dis.* 90:607.
6. Fireman, P., and J. Kumate. 1969. Effect of measles vaccine on immunologic responsiveness. *Pediatrics.* 43:264.
7. Kantor, F. S. 1975. Infection, anergy and cell mediated immunity. *N. Engl. J. Med.* 292:629.
8. Finkel, A., and P. B. Dent. 1973. Virus-leukocyte interactions: relationship to host resistance in virus infections in man. In *Pathobiology Annual*. H. L. Loachimed, editor. Appleton-Century-Crofts, New York. pp. 47.
9. Bloomfield, A. L., and J. Mateer. 1919. Changes in skin sensitiveness to tuberculin during epidemic influenza. *Am. Rev. Tuberc. Pulm. Dis.* 3:166.
10. Lachmann, P., and D. K. Peters, editors. 1982. *Clinical Aspects of Immunology*. Blackwell Scientific Publications, Oxford. 4th Edition, Vol. 2.
11. Whittle, H. C., A. Bradley-Moore, A. Fleming, and B. M. Greenwood. 1973. Effects of measles on the immune response of Nigerian children. *Arch. Dis. Child.* 48:753.
12. Zweiman, B., D. Pappagianis, H. Maibach, and E. A. Hildreth. 1971. Effect of measles immunization on tuberculin hypersensitivity and in vitro lymphocyte reactivity. *Int. Arch. Allergy Appl. Immunol.* 40:834.
13. Finkel, A., and P. B. Dent. 1973. Abnormalities in lymphocyte proliferation in classical and atypical measles infection. *Cell. Immunol.* 6:41.
14. Whittle, H. C., J. Dossetor, A. Oduloju, D. M. Bryceson, and B. M. Greenwood. 1978. Cell-mediated immunity during natural measles infection. *J. Clin. Invest.* 62:678.
15. Hirsch, R. L., F. Mokhtarian, D. E. Griffin, B. R. Brooks, J. Hess, and R. T. Johnson. 1981. Measles virus vaccination of measles seropositive individuals suppresses lymphocyte proliferation and chemotactic factor production. *Clin. Immunol. Immunopathol.* 21:341.
16. Anderson, R., A. R. Rabson, R. Sher, H. J. Koornhof, and D. Bact. 1976. Defective neutrophil motility in children with measles. *J. Pediatrics.* 89:27.
17. Osler, W. 1904. *The Principles and Practice of Medicine*. D. Appleton and Company, New York. Fifth ed. p. 88.
18. Beck, V. 1962. Measles virus epidemic in Greenland. *Am. J. Dis. Child.* 103:252.
19. Hutkins, G., and C. Janeway. 1947. Observations on the relationship of measles and remissions in the nephrotic syndrome. *Am. J. Dis. Child.* 73:242.
20. Blumberg, R., and H. Cassady. 1947. Effect on measles on the nephrotic syndrome. *Am. J. Dis. Child.* 73:151.
21. Notkins, A. L., J. E. Mergenhagen, and P. J. Howard. 1970. Effects of virus infections on the function of the immune system. *Am. Rev. Microbiol.* 24:525.
22. Wheelock, E. F., and S. T. Toy. 1973. Participation of lymphocytes in viral infections. *Adv. Immunol.* 16:123.
23. Woodruff, J. E., and J. J. Woodruff. 1975. T lymphocytes interaction with viruses and virus-infected tissues. *Prog. Med. Virol.* 19:120.
24. Bloom, B. R., A. Senick, G. Stoner, G. Ju, M. Nowakowski, S. Kano, and L. Jimenez. 1977. Studies of the interactions between viruses and lymphocytes. *Cold Spring Harbor Symp. Quant. Biol.* XLI:73.
25. Osunkoya, B. O., A. R. Cooke, O. Ayeni, and T. Adejunio. 1976. Studies on leukocyte

- cultures in measles. I. Lymphocytes transformation and giant cell formation in leukocyte cultures from clinical cases of measles. *Arch. Gesamte Virusforsch* 44:313.
26. Wrzos, H., J. Kulczycki, Z. Laskowski, D. Maracz, and W. J. Brzosko. 1979. Detection of measles virus antigens in peripheral lymphocytes from patients with subacute sclerosing panencephalitis. *Arch. Virol.* 60:291.
 27. Robbins, S. J., H. Wrzos, A. L. Kline, R. B. Tenser, and F. Rapp. 1981. Rescue of a cytopathic paramyxovirus from peripheral blood lymphocytes in subacute sclerosing panencephalitis. *J. Infect. Dis.* 143:396.
 28. Horta-Barbosa, L., R. Hamilton, B. Wittig, D. A. Fucillo, J. L. Sever, and M. L. Vernon. 1971. Subacute sclerosing panencephalitis: isolation of suppressed measles virus from lymphonode biopsies. *Science (Wash. DC)*. 173:840.
 29. White, R. G., and J. F. Boyd. 1973. The effects of measles virus on the thymus and other lymphoid tissues. *Clin. Exp. Immunol.* 13:343.
 30. Sullivan, J. L., D. W. Barry, S. J. Lucas, and P. Albrecht. 1975. Measles virus infection of human mononuclear cells. I. Acute infection of peripheral blood lymphocytes and monocytes. *J. Exp. Med.* 142:773.
 31. Joseph, B. S., P. W. Lampert, and M. B. A. Oldstone. 1975. Replication and persistence of measles virus in defined subpopulations of human leukocytes. *J. Virol.* 16:1638.
 32. Huddleston, J. R., P. W. Lampert, and M. B. A. Oldstone. 1980. Virus-lymphocyte interactions: infection of Tg and Tm subsets by measles virus. *Clin. Immunol. Immunopathol.* 15:502.
 33. Jacobson, S., and H. McFarland. 1982. Measles virus persistence in human lymphocytes: a role for virus induced interferon. *J. Gen. Virol.* 63:351.
 34. Jacobson, S., and H. McFarland. 1984. Measles virus infection of human peripheral blood lymphocytes: importance of the OKT4⁺ T cell subset. In *Molecular Biology of Negative Strand Viruses*. R. Compans and D. Bishop, editors. Academic Press, New York. In press.
 35. Casali, P., J. G. P. Sissons, R. S. Fujinami, and M. B. A. Oldstone. 1981. Purification of measles virus glycoproteins and their integration into artificial lipid membranes. *J. Gen. Virol.* 54:161.
 36. Casali, P., J. G. P. Sissons, M. J. Buchmeier, and M. B. A. Oldstone. 1981. Generation of human cytotoxic lymphocytes by virus: viral glycoproteins induce nonspecific cell-mediated cytotoxicity without release of interferon in vitro. *J. Exp. Med.* 154:840.
 37. Oldstone, M. B. A., R. S. Fujinami, A. Tishon, D. Finney, H. C. Powell, and P. W. Lampert. 1983. Mapping of the major histocompatibility complex and viral antigens on the plasma membrane of a measles virus infected cell. *Virology*. 127:426.
 38. Boyum, A. 1968. Separation of leukocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest.* 21 (suppl. 97):1.
 39. Dahl, H. 1973. A micro assay for mouse and human interferon. II. Dose-response in different cell-virus systems. *Acta Pathol. Microbiol. Scand. Sect. B Microbiol.* 18:359.
 40. Harfast, B., J. R. Huddleston, P. Casali, T. Merigan, and M. B. A. Oldstone. 1981. Interferon acts directly on human B lymphocytes to modulate immunoglobulin synthesis. *J. Immunol.* 1217:2146.
 41. Rice, G. P. A., P. Casali, and M. B. A. Oldstone. 1983. A new solid-phase enzyme-linked immunosorbent assay for measles virus specific antibodies. *J. Infect. Dis.* 147:1055.
 42. Casali, P., and G. Trinchieri. 1984. Natural killer cells in viral infection. In *Concepts in Viral Pathogenesis*. A. Notkins and M. B. A. Oldstone, editors. Springer-Verlag New York Inc., New York. In press.
 43. Perussia, B., S. Starr, S. Abraham, V. Fanning, and G. Trinchieri. 1983. Human

natural killer cells analysed by B73.1, a monoclonal antibody blocking Fc-receptor function. I. Characterization of the lymphocyte subset reactive with B73.1. *J. Immunol.* 130:2133.

44. Oldstone, M. B. A., Y. N. Sinha, P. Blount, A. Tishon, M. Rodriguez, R. von Wedel, and P. W. Lampert. 1982. Virus-induced alterations in homeostasis: alterations in differentiated functions of infected cells in vivo. *Science (Wash. DC)*. 218:1125.
45. Borysiewicz, L. K., S. Morris, J. D. Page, and J. G. P. Sissons. 1983. Requirements for in vitro generation of human cytomegalovirus specific cytotoxic T lymphocyte lines. *Eur. J. Immunol.* 130:804.
46. Lucas, C. J., M. L. Achem, M. Galama, and J. Ubels-Pastma. 1979. Lymphocytes and measles virus in virus-lymphocyte interactions. M. Proffitt, editor. Elsevier-North Holland, New York. p. 243.
47. Galama, J., J. C. Ubels-Postma, A. Vos, and C. J. Lucas. 1980. Measles virus inhibits acquisition of lymphocyte functions but not established effector functions. *Cell. Immunol.* 50:405.
48. Valdimarsson, H., G. Agnarsdottir, and P. J. Lachmann. 1975. Measles virus receptors on human T lymphocytes. *Nature (Lond.)*. 255:554.
49. Clinical Concepts of Infectious Diseases. 1978. L. Cluff and J. Johnson, editors. Williams & Wilkins Co., Baltimore. p. 410.
50. Fireman, P., G. Friday, and G. Kumate. 1969. Effects of measles vaccine on immunological responsiveness. *Pediatrics*. 43:264.
51. Asklerov, V. F., and M. K. Voroshilova. 1968. Izuchenie vliyaniya kaevol infektsii, i privivki zhirol protivokorevoi vaksiny na sostoyaniye gumoral' nogo immuniteta k poliomiellitu u detey. *Vopr. Virusol.* 1:42.
52. Wilson, A. B., D. N. Planterose, J. Nagington, J. R. Park, R. D. Barry, and R. R. A. Coombs. 1976. Influenza A antigens on human lymphocyte in vitro and probably in vivo. *Nature (Lond.)*. 259:582.
53. Pelton, B. K., B. Imrie, and A. Denman. 1977. Susceptibility of human lymphocyte populations to infection by herpes simplex virus. *Immunology*. 32:803.