Cellular Immunity to Measles Virus Infection: Assay of Human Immune Interferon and Lymphocyte Transformation

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CELLULAR IMMUNITY TO MEASLES VIRUS INFECTION: ASSAY OF HUMAN IMMUNE INTERFERON AND LYMPHOCYTE TRANSFORMATION

BY

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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In memory of my father,

Dr. George Alexander Berman

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ABBREVIATIONS

PHA - Phytohemagglutinin

CMI - Cell mediated immunity

Ig - Immunoglobulin

LPS - Lipopolysaccharide

NDV - Newcastle disease virus

HSV - Herpes simplex virus

SI - Stimulation index

IF - Interferon

GMT - Geometric mean titer

A B S T R A C T

Lymphocyte transformation and "immune" interferon production were used to study cellular immunity to measles virus in 16 adult and 9 children patients. Rubeola complement fixation antigen was employed in all *in vitro* assays. Lymphocyte response of children following vaccination with live attenuated measles vaccine was found to be significantly higher than before vaccination. There was also a significant increase of immune interferon production two weeks after vaccination among 9 seronegative adults. However, with one exception, live attenuated measles vaccine was not successful in eliciting humoral immunity in seronegative adults. Moreover, reimmunization of 4 adults had no effect on either humoral or cell mediated immune response. Detection of both humoral and cellular immunity was achieved only following atypical measles.

I N T R O D U C T I O N

Since the introduction of measles vaccine in 1963, a significant decline in the measles morbidity occurred in the United States. Nevertheless, measles still remains a significant public health problem. After an all-time low of 22,094 reported cases of measles in 1974, the number increased dramatically to 41,126 in 1976 and to 54,847 in 1977, the highest total since 1971 (Orenstein et al, 1978). Although vaccine failures were not the primary cause of repeated measles epidemics in the United States, they usually accounted for over 10% of the total cases (Currier et al, 1972; Wyll et al, 1971; Measles Surveillance, 1973). Similar findings were reported in Winnipeg, Manitoba (Barsky, 1974). From January to June of 1973, 688 cases of measles were reported, 73 of which occurred in previously immunized children.

It had been suggested that certain populations, specifically American Indians and Eskimos, were unusually susceptible to measles. With the measles vaccine reaction as a model for the natural disease, several American Indian populations were studied (Black et al, 1971). It was found that the average fever in the Indian population was higher compared to those in standardized series of studies among Caucasians. In the 1973 epidemic in Manitoba, there were two reported measles associated deaths in Winnipeg and both victims were Canadian Indians (Personal Communication with Department of Health and

Social Development, 1978).

The purpose of this investigation was to study the host responses during measles infections in both Canadian Indians and Caucasians, specifically following measles immunization with live attenuated vaccine. Special attention was given to measles seronegative individuals in order that their susceptibility to the disease might be determined. The antibody status of donors was determined by the hemagglutination inhibition antibody titers. The cell mediated immune response was determined by lymphocyte transformation and "immune" interferon production before and after immunization. Another objective was to evaluate the measles vaccination, using assays of cell mediated immunity as well as the usual serological tests; and furthermore, to correlate the humoral and cell mediated immunity with respect to each other and to the immunity of donors.

L I T E R A T U R E R E V I E W

IN VITRO STUDIES OF IMMUNITY TO MEASLES VIRUS

It has become increasingly apparent that cell mediated immunity may play an important role in host response to infection with viruses, particularly those maturing at membranes on the cell surface (Glasgow, 1970). Good and Zak (1956) observed that children suffering from hypoagammaglobulinaemia survived measles infection and acquired life-long immunity; whereas fatal measles infection was well documented in children with evidence of lymphocyte hypofunction (Enders et al, 1959; Meadow, 1969).

Due to the lack of suitable and reproducible assay for in vitro study of cell mediated immunity, the immunity to measles virus and other related viruses has been generally assessed by production of antibodies. Heffner and Schluederberg (1967) characterized antibody response to myxoviruses in terms of species of immunoglobulins (Ig), separated by density gradient centrifugation. Thus, 19S (IgM) antibodies were indicative of recent exposure, while 7S (IgG) antibodies of past exposure, to measles virus.

The production of antibodies has been further characterized. It had been observed that the medipest viruses, namely measles, canine distemper and rinderpest viruses, contained common nucleocapsid components which could not be distinguished by using immunologic techniques (Orvell and Norrby, 1974). Studies with measles virus revealed

that antibodies against nucleocapsid dominated quantitatively over antibodies against envelope structure in convalescent sera (Norrby and Gollmar, 1972). Similar findings have been made on sera from individuals immunized with live measles vaccine.

On the other hand, the relative protective value of inactivated measles vaccine has been found to be markedly inferior to live attenuated vaccines. Following immunization with three doses of this vaccine, protection against the disease, after being challenged with live measles virus, was of relatively short duration and sometimes resulted in the development of aberrant reactions (Fulginiti et al, 1967; Chatterji and Mankad, 1977). This failure of inactivated measles vaccine to prevent infection was explained by the absence of the envelope components in the vaccine (Norrby et al, 1975). As a result of these experiments further use of inactivated vaccine has been discouraged.

The loss of delayed cutaneous hypersensitivity to tuberculin during measles infection was first described in 1908 by Von Pirquet, and has been confirmed both during natural infection and after live attenuated measles immunization (Starr and Berkovich, 1964). To determine the mechanism responsible for the depression of delayed cutaneous hypersensitivity, efforts were focused on in vitro correlates of cell mediated immunity. One of the tests used was the H^3 thymidine incorporation of lymphocytes stimulated by phytohemagglutinin (PHA).

One study showed that lymphocyte responsiveness to PHA stimulation was impaired in patients with measles infection (Finkel and Dent, 1973); another, however, showed an impairment of lymphocyte responsiveness to stimulation with candida antigen but not with PHA (Munyer et al, 1975). The apparent impairment of lymphocyte response to PHA was attributed to the increase in spontaneous DNA synthesis, since the absolute counts per minute of H^3 thymidine incorporation were essentially unchanged.

Previous studies had also yielded conflicting data concerning the effect of in vitro addition of measles virus to PHA-stimulated lymphocytes. Zweiman reported that addition of autoclaved noninfective measles virus to lymphocytes markedly inhibited the cellular proliferation induced by PHA. Indeed, this suppression was greater than that exerted by live virus (Zweiman, 1972). But Sullivan and others maintained that the addition of live measles virus and not UV-irradiated or heat inactivated virus was responsible for this inhibition. In fact, this inhibitory effect was seen in lymphocytes of both measles immune and susceptible subjects (Sullivan et al, 1975).

Further studies were focused on the interaction of measles virus and lymphoid cells in order to achieve a better understanding of the pathogenesis of measles and its effect on the immune system. These included

observations of extensive formation of giant cells of thymocytes within the medulla and the cortex of the thymus gland. These giant cells were comparable to syncytia in nonlymphoid cell cultures as a result of measles virus infection. In autopsies of children with severe measles of more than four days duration, a loss of discernible cortex from the thymus was found. In less severe cases, the damaged cortex took at least one month to recover. These destructive changes in the thymus could lead to a decreased lymphocyte count in the peripheral blood (White and Boyd, 1973).

Similar findings were reported in monkeys (Nii and Kamahora, 1964). Furthermore, using the fluorescent antibody technique on frozen sections of spleen of infected monkeys revealed a large amount of viral antigen in the germinal centre as well as in the giant cells. From this, Yamanouchi and associates concluded that the reticular cells of monkeys were the main target of measles virus growth and that the giant cells were formed as a result of virus growth in situ (Yamanouchi et al, 1973).

Osunkoya and co-workers also observed giant cell formation in PHA-stimulated lymphocyte cultures from the acute phase of measles infection. But the giant cells disappeared after the third day in culture; hence suggesting that it might be the result of a lethal cytopathic

effect of the virus on the proliferating lymphoid cells. Moreover, the presence of giant cells in proliferating PHA-stimulated cultures and their absence in control unstimulated cultures indicated that viral replication proceeds better in proliferating than in resting lymphoid cells. Furthermore, the formation of giant cells in measles PHA cultures was not associated with depression of lymphocyte transformation (Osunkoya et al, 1973, 1974).

Measles virus was also found to have reduced the antibody formation against 2,4 Dinitrophenyl in mice, primarily as a result of the suppression of the T helper cell function and possibly, though to a lesser extent, also the B cell function. This T cell suppression, nevertheless, was short-lived and apparently was dependent on the replication of virus in the host, since heat inactivated virus did not suppress T cell function (McFarland, 1974).

With new approaches to studying in vitro cell mediated immunity to viral agents, the relative roles of humoral and cellular immunity with respect to resistance and recovery from infection were being reconsidered. In a study of serological responses in gnotobiotic dogs infected with canine distemper virus, it was found that antibody titers varied inversely with the severity of disease. Recovered dogs demonstrated the highest titers of antibody activity in their sera (Krakowka et al, 1975).

Experiments with Cercopithecus monkeys demonstrated cellular immunity by lymphocyte mediated colony-forming

inhibition after primary but not secondary inoculation with live measles virus. The cell mediated immune response became undetectable as early as ten weeks after infection (Rustigian et al, 1975).

On the other hand, using a lymphocyte mediated cytotoxicity assay Labowskie and his colleagues (1974) observed a measles specific cytotoxic reaction in seropositive individuals with previous measles history.

Chiba and associates (1974) even obtained measles-specific cytotoxicity with all measles seropositive individuals regardless of measles history.

Furthermore, Graziano and Ruckdeschel demonstrated cellular immunity to measles using H^3 thymidine incorporation. The cellular immunity was determined not only in seventeen seropositive individuals with measles history but also in two seronegative pediatric residents who had been frequently exposed to measles. These observations suggested that the cellular responsiveness to measles complement fixation antigen was an *in vitro* correlate of their clinical protection against measles (Ruckdeschel et al, 1975; Graziano et al, 1975).

In 1977 Kreeftenberg and Loggen also showed that measles specific immunity could be measured with the lymphocyte stimulation test. Following the vaccination with live measles vaccine, all seropositive children tested showed positive responsiveness to measles antigen; whereas seronegative children did not react with measles antigen.

IN VITRO TESTS FOR CELLULAR IMMUNITY TO OTHER VIRUSES

A study of cell mediated immunity (CMI) measured by lymphocyte transformation and ^{51}Cr-release microassay to other viruses showed that a correlation existed between CMI and antibody titer. CMI was detected in patients sero-positive to rubella virus (Steele et al, 1973; Lalla et al, 1973), varicella-zoster virus (Jordan and Merigan, 1974), herpes simplex virus (Rusmussen et al, 1974; Haahr et al, 1976), mumps virus (Rola-Pleszczynski et al, 1976) and influenza antigen following immunization (Ruben et al, 1973); but it was not detected in patients seronegative to the viruses mentioned above.

CMI to mumps virus was found to correlate with delayed type skin hypersensitivity using the lymphocyte transformation assay (Smith et al, 1972) and the production of macrophage inhibition factor (Jurgensen et al, 1973), but there was no correlation found using the lymphocyto-toxicity assay (Rola-Pleszczynski et al, 1976).

The cell mediated immune response measured by H^3 thymidine incorporation and the production of macrophage inhibition factor was greater in magnitude and duration following natural rubella than following rubella vaccination (Lalla et al, 1973; Honeyman et al, 1974). The peak of the cell mediated response, determined by ^{51}Cr-release, occurred seven to fourteen days following rubella vaccination and slowly declined thereafter (Steele et al, 1974).

It was also shown that following congenital rubella cellular immunity was not detected using ^{51}Cr-release microassay. However, this lack of cellular immunity might be reactivated with natural rubella infection later in life. Rubella vaccine was not found to be an effective stimulant of CMI in the presence of antibodies (Fuccillo et al, 1974).

Lymphocyte responsiveness to PHA was shown to be transiently depressed eleven to twenty days after rubella vaccination (McMorrow et al, 1974); but others did not observe a depressed response to PHA following rubella vaccination (Lalla et al, 1973; Fuccillo et al, 1974).

INTERFERONS

Interferons are a group of soluble, nontoxic, antiviral proteins, synthesized by most cells of vertebrate origin in response to viral infections and certain other agents. They do not sediment at 100,000 g for 24 hours. They are inactivated by trypsin but not by lipases or nucleases. The antiviral action of interferon is inhibited by actinomycin D (Finter, 1973).

Interferons were described for the first time in 1957 by Isaacs and Lindenmann; but as yet there has been no entirely satisfactory explanation for their potent antiviral effect. One of the reasons for this seems to lie in the impressive biological activity of interferon. A recent estimate of the specific antiviral activity of human interferon was at least 2×10^8 reference units per mg of protein, and it may be ten to one hundred times more potent (Friedman, 1977). As a consequence, interferon preparations exhibiting very large amounts of antiviral activity turn out to contain extremely small amounts of interferon.

Two major antigenic species of human interferon can be distinguished on the basis of specific neutralization of biological activities and binding to antibody-affinity columns. One interferon species, termed F, is produced by diploid fibroblasts in culture as well as by many other human cells of nonlymphoid origin. Another

antigenic species, termed Le, forms the bulk of interferon elaborated in primary cultures of leucocytes from peripheral blood. However, crude preparations of leucocyte interferon also contain a small fraction of interferon with F antigenic specificity (Havell et al, 1975).

When crude leucocyte interferon was analyzed on sodium dodecyl sulphate-polyacrylamide gel electrophoresis, two forms were obtained; namely, a slower migrating leucocyte interferon species (Le_s) with a molecular weight of approximately 23,000 and a faster migrating leucocyte interferon species (Le_f) with a molecular weight of 17,500 (Havell et al, 1977). On sodium dodecyl sulphate-polyacrylamide gel human fibroblast interferon activity formed a single homogeneous peak with a molecular weight of approximately 20,000 (Vilcek et al, 1977). In addition, human T lymphocytes produce an "immune" interferon in response to antigens and mitogens but it differs from Le and F interferon species. However, these mitogens do not stimulate interferon production in non-immunocompetent cells.

INTERFERON PRODUCTION AS A TEST FOR IN VITRO CELLULAR IMMUNITY

The finding that interferon was produced by lymphoid cells in vitro on an immune specific basis seemed to indicate that it might act as one of the mediators of cellular immunity (Glasgow, 1966; Green et al, 1969). Production of interferon was studied with many viruses and antigens. Experiments by Youngner and Salvin (1973) using Mycobacterium tuberculosis, BCG strain showed that interferon produced in vivo in BCG-infected mice by the inoculation of a specific antigen, Old Tuberculin, was different from the viral inhibitors produced in hypersensitive mice in response to nonspecific stimuli. They designated type I interferon as interferon produced in BCG-infected or control mice as well as interferon produced in mouse cell cultures exposed to nonspecific stimuli such as lipopolysaccharide, Statolon, Poly I:C or Newcastle disease virus.

The designation type II interferon was used for the inhibitor elicited in hypersensitive mice inoculated with a specific antigen. In this case the BCG-infected mice were challenged with Old Tuberculin. Type II interferon in contrast to type I interferon was unstable at pH 2 and stable at 56^{o}C. Type I interferon was active in primary guinea pig kidney cultures whereas

type II was not. The activity of type II interferon was not neutralized by antibodies against type I interferon. In all other respects, however, type II interferon met the criteria required to classify a viral inhibitor as interferon. Evidence was also presented with regard to the similarity between properties of migration inhibitory factor and type II interferon (Youngner and Salvin, 1973).

Studies on mouse immunocompetent cell types, stimulated by plant mitogens, suggested that spleen T but not B cells were required for the interferon production, though macrophages were thought to enhance this response (Stobo et al, 1974). The stability of interferon at pH 2, however, was not tested. Similar experiments with mice were conducted by another group of investigators (Neumann and Sorg, 1978). According to their results, the interferon activity was released by macrophages following induction by lymphokines, while T cells were the primary producers of migration inhibitory factor.

Further studies on mice showed that in vivo administration of interferon before antigenic stimulation significantly suppressed antibody response to both sheep red blood cells, a T cell-dependent antigen, and lipopolysaccharide, a T cell-independent antigen (Brodeur and Merigan, 1975). Maximum suppression was produced between 4 and 48 hours before antigenic stimulation. However, interferon treatment after the antigenic stimulation was found to have significantly increased the

antibody response to sheep red blood cells. The mechanism of this phenomenon is, unfortunately, not yet clear.

During the course of human interferon production studies it was noted that reimmunization with live vaccinia virus caused an increase in interferon production in vitro in their white blood cell cultures four to seven weeks later (Epstein et al, 1972). Macrophages were reported to have greatly increased the amount of interferon produced. This increase in interferon production in vitro was suggested to be a direct consequence of a specific immunological response to vaccinia antigen. No significant interferon production was noted in cultures prepared from non-vaccinated donors or in vaccinia antigen control-treated cultures from vaccinated donors. Falcoff suggested the term "immune" interferon for human interferon synthesized by immunocompetent cells and the term "virus" interferon for the inhibitor induced by virus infection of any other kind of human cell (Falcoff, 1972).

Rusmussen and her associates also found that macrophage-lymphocyte cultures were required for optimum interferon production. The increase in interferon production by lymphocytes reached a maximum between two and six weeks following herpes virus hominis disease. This was explained as a response to active disease occasioned by previous sensitization as evidenced by lymphocyte transformation and presence of antibody. It

is important to note that recurrence of lesions was more frequent in subjects who failed to produce interferon within the two to six week period after disease when compared to those who were able to do so. (Rusmussen et al, 1974).

Immune specific interferon to varicella-zoster antigen was not detected using both macrophage-lymphocyte and ficoll-hypaque cell cultures (Jordan and Merigan, 1974). Using the latter method of lymphocyte preparation, only non-specific interferon production was seen in presumably nonimmune children. Further evidence that this interferon was produced in a nonspecific manner was given by its stability at pH 2.

The interferon production in vitro as a result of herpes simplex virus stimulation was shown to be dependent on T cells. The memory for the immune specific release of interferon also appeared to be carried by the T lymphocytes rather than macrophages (Valle et al, 1975a). This interferon was characterized and found to be unstable at pH 2 and at 56^{o}C. Rabbit anti-human leucocyte interferon serum was not active against immune interferon. However, when a more heterogeneous cell population was used as in ficoll-hypaque cell cultures, a slight cross-reaction with antiserum to leucocyte interferon occurred (Valle et al, 1975b).

Haahr then studied the cellular immunity to herpes simplex virus using different methods of preparing mononuclear cell cultures. Significant differences in the interferon production were found between donors with a history of herpes labialis and those with no detectable antibody. Immune interferon production occurred only with autochthonous macrophage and column purified lymphocyte cultures. Interferon produced by ficoll-hypaque purified mononuclear cultures was type I or "virus" interferon and its production was unrelated to immune status (Haahr et al, 1976).

It was observed that the interferon production as a result of rubella virus stimulation was 12-14 times greater in cultures of lymphocytes from individuals immune to rubella than from individuals susceptible to rubella (Buimovici-Klein et al, 1977). The preliminary results of the rubella virus-induced interferon were consistent with the recognized properties of type II or "immune" interferon. Its activity decreased by 50-60% after heating for one hour at 56^{o}C and there was a loss of activity by 30-40% following exposure to pH 2. (Table I)

Table 1. Characteristics of Mouse and Human Interferon.

Name	INTERFERON Source	Inducer	STABILITY at pH2 (24 hrs at 4°C)	HEAT STABILITY (1 hr at 56°C)
Type I	Mouse Cell Cultures, BCG,** infected or control mice.	nonspecific (e.g. LPS,* NDV+)	yes	no
"virus"	any Human cell	virus infection	yes	no
Type II	Hypersensitive Mice	specific antigen (e.g. OT)++	no	yes
"immune"	Human immunocompetent cells previously sensitized	specific antigen, PHA***	no	no

* lipopolysaccharide
\+ Newcastle disease virus
** Mycobacterium tuberculosis, strain BCG
++ Old tuberculin
*** Phytohemagglutinin

MEASLES VIRUS AS AN INTERFERON INDUCER

Very little has been reported on interferon stimulated by measles virus in human cell cultures. In 1961 DeMaeyer and Enders observed that fluids from human amnion cultures, infected with measles virus, showed inhibitory activity. This activity depended upon the presence of an interferon-like agent which they called measles interferon. Moreover, interferon was detected in the serum of over 80% of children examined six to eleven days after primary measles vaccination (Petralli et al, 1965). This was consistent with the hypothesis that interferon helps to prevent clinical manifestations of measles, even when attenuated measles vaccine was given three days after exposure to the virus.

Several viruses were studied as interferon inducers in leucocyte cultures of children vaccinated against measles (Trubina et al, 1972); but only measles virus was found to stimulate significant amounts of interferon. It was therefore proposed that the "immunological memory" of leucocytes was activated by the challenging measles virus and, as a result, a specific production of interferon was induced. This interferon production persisted through the observation period of two years.

Both virulent and avirulent (Attenuvax vaccine by Merck, Sharp and Dohme, Haarlem, The Netherlands) strains of measles virus were also tested for their interferon-inducing capacity in human lymphoblast cells. The virulent strain induced only minimal amounts of interferon while the attenuated strain turned out to be a much better interferon producer. Nevertheless, the amount was still ten times lower than the one which could be regularly obtained using human diploid fibroblasts (Volckaert-Vervliet and Billiau, 1977).

MATERIALS AND METHODS

I. Study Population

The following groups were studied:

1. Nine healthy nursing and medical students screened for measles susceptibility and requiring measles immunization. Their measles history was unknown.

2. Seven healthy Canadian Indian children with negative measles history who were undergoing live measles immunization at the community Health Services in Winnipeg.

3. Two healthy children, following a course of atypical measles infection.

4. Four healthy adults undergoing reexposure to measles virus with live measles vaccine.

5. Seven healthy adult volunteers with a previous measles history of at least one year.

All donors to be immunized were vaccinated with live attenuated measles vaccine "Attenuvax - more attenuated Enders' line of Edmonton strain "prepared in cell cultures of Chick Embryo by Merck, Sharp & Dohme.

II. Collection of Samples and Lymphocyte Isolation

Ten ml of blood was drawn from children and 20 ml from adults. Two ml and three ml respectively were collected for serological tests. The remaining blood was aliquoted into heparinized tubes and diluted 1:2 with

RPMI 1640 medium (Flow Laboratories cat. no. 2-068M) containing penicillin, streptomycin and L-glutamine. This mixture was layered onto a ficoll-isopaque (Pharmacia Fine Chemicals, Nyegaard & Co.) gradient (Boyum, 1968) in glass test tubes and centrifuged at room temperature for 25 minutes at 400 x g. Following centrifugation, the lymphocyte rich layer above the ficoll-isopaque was aspirated with a pasteur pipette. The cells were washed three times with RPMI 1640 medium. The pellet was resuspended in RPMI 1640 supplemented with 20% agamma fetal calf serum (FCS) (Flow Laboratories) and the cells were counted. One million lymphocytes in one ml volumes were added to Falcon culture tubes (12 x 75 mm).

III. Stimulation of Lymphocytes with Antigens and Mitogens

A. Antigens and Mitogens

1. Rubeola antigen - complement fixation antigen, (Flow Laboratories) grown in African Green Monkey kidney cell line. This was used in 0.05 ml aliquots per lymphocyte culture. The final dilution in culture was 1:20 and 1:100.

2. Rubeola antigen control - complement fixation control (Flow Laboratories). The final dilution in lymphocyte cultures was 1:20. It was included in all tests as a control to show that the response to rubeola antigen was specific and not a result of stimulation by cell culture in which the antigen was prepared.

3. Rubella antigen - complement fixation antigen (Flow Laboratories). The final dilution in culture was 1:40. Rubella antigen was used to further confirm the specificity of the response to measles virus in both lymphocyte transformation and interferon production assay.

4. Phytohemagglutinin - PHA-P (Difco Laboratories)

PHA was used at a concentration of 21.25 µg per culture as a positive control in lymphocyte stimulation studies.

Stimulants were added at the initiation of lymphocyte cultures and were incubated at 37°C and 5% CO_2 for six days.

B. Thymidine Incorporation

For the final 18 hours of incubation 1 µCi of H^3 thymidine (specific activity 2 Ci/mmole, aqueous solution) (New England Nuclear) was added into each culture tube. The incorporation of thymidine was arrested by addition of cold saline. Lymphocytes were washed onto glass fiber filter papers (Whatman GF/C, 2.4 cm) with saline, followed by washes with cold 5% trichloroacetic acid, 10% trichloroacetic acid, distilled water and methanol. Filter papers were put into scintillation vials and dried overnight. The following day they were suspended in 10 ml of scintillation fluid (19 g PPO* + 380 mg POPOP** per one gallon of toluene) and counted in a Beckman DPM-100 Liquid Scintillation counter for ten minutes.

The results were formulated as a Stimulation Index (SI), defined as mean counts per minute of cultures

* PPO - 2,5 Diphenyloxazole

**POPOP - 1, 4-Bis[2-(5-Phenyloxazolyl)]-Benzene

incubated with antigen or mitogen divided by mean counts per minute of control cultures.

IV. The Interferon Assay

A. Human Foreskin Fibroblast (HFF) Monolayers

Human foreskins were collected every week in 5 ml Eagle's Minimum Essential Medium (MEM) (Flow Laboratories, cat. no. 1A-020) and "double strength" of antibiotics (penicillin 500 IU/ml, Fungizone 5.0 µg/ml, gentamicin sulphate 80 µg/ml). They were kept at $4^{o}C$ before processing.

Ten to fifteen foreskins were pooled in an erlenmeyer flask and washed three times with phosphate buffer saline (PBS). Thirty ml of Versene-trypsin solution (PBS + 0.02% EDTA + 0.25% trypsin) was added to the flask and was incubated at $37^{o}C$ on a magnetic stirrer. After 20 minutes, the first trypsin solution containing cells was discarded. Subsequent aliquots were centrifuged in two ml ice cold FCS at 1200 rpm for 15 minutes. The pellet was resuspended in 15 ml MEM supplemented with 20% FCS and incubated at $37^{o}C$ in 75 cm^2 Falcon tissue culture flask. Fresh trypsin solution was added to the trypsinization flask. Additional three or four harvests were performed, each time making a new flask of cells (Merigan, 1971).

The cells became attached to the flask in four to five days in 5% CO_2 and 37°C. The mediumwas then replaced by growth medium (MEM + 10% FCS) and cells were observed until they became confluent (approximately ten days). The confluent monolayer could be maintained for seven to ten days on maintenance media (MEM + 2% FCS). The HFF were propagated by splitting them 1:3 with Versene-trypsin solution and suspending them in growth media. The cells were periodically tested on antibiotic free media for bacterial contamination. They were used up to 20 passes.

All MEM media were supplemented with L-glutamine, penicillin (250 IU/ml), Fungizone (2.5 µg/ml) (all from Flow Laboratories) and gentamicin sulphate (40 µg/ml) (Schering Co. Ltd. Canada).

B. Viruses

Newcastle Disease Virus (NDV), Hickman Delaware Strain, was provided by Bryce Larke, Head of Provincial Virus Laboratory, Edmonton, Alberta. It was propagated in ten days old embryonated eggs by Miss Doris Schulz. The inoculum was injected with a syringe into the allantoic cavity and the eggs were incubated at 37°C for 48 hours. After chilling the eggs, the allantoic fluid containing the virus was harvested. The virus was titrated by plaque assay on chick embryo fibroblast cells. It was stored at -70°C in one ml aliquots.

Vesicular Stomatitis Virus (VSV), HR strain, was kindly provided by Dr. D.V. Cormack from the Cancer Research and Treatment Foundation. The virus was grown in L-cells and titrated by plaque reduction assay on HFF cells. It was stored in liquid nitrogen in 0.5 ml aliquots.

C. Laboratory Standard IF

One week old monolayers of HFF, in 150 cm^2 tissue culture flask, were washed with 20 ml of serum-free MEM (diluent). The cells were challenged with 5 x 10^7 plaque forming units of NDV in 5 ml diluent for one hour. An additional 15 ml of diluent was added and the flask was incubated at 37°C and 5% CO_2 for a total of 48 hours. The MEM containing IF was then harvested and clarified by centrifugation. The pH was lowered to two with 1N HCl and held at 4°C for five days to destroy any remaining virus. Following this, the pH of the preparation was raised to seven with 1N NaOH. Standard IF was titered by plaque assay and 0.5 ml aliquots were frozen at -70°C (Merigan, 1971).

D. Assay for Interferon (IF)

The supernatants, containing IF, produced in vitro by the patient's lymphocytes, were collected following six days of incubation and frozen at -70°C before assay-

ing for IF. The human IF was assayed by employing HFF monolayers and a VSV plaque inhibition assay (Merigan, 1971). Samples were diluted serially in half log dilutions (to make 1:10 dilution 0.29 ml of sample was mixed with 2.61 ml of diluent; 1:30 dilution and all subsequent half log dilutions were made by mixing 0.9 ml of previous dilution with 2 ml of diluent). These dilutions were poured in 2 ml volumes onto HFF monolayers in Costar 6-well (38 mm) plates. After 24 hours the monolayers were washed with serum-free MEM and approximately 50-80 plaque forming units of VSV in 0.05 ml volumes were added to each well. After a 30 minute absorption period the monolayers were covered with two ml of nutrient agar overlay (50 ml of "double concentration" maintenance MEM, at 37^{o}C, was mixed with melted 2% agar just prior to use). In approximately 44 hours the monolayers were stained with 0.02% neutral red. Plaques appeared as localized areas of unstained cells in a red background.

E. Interferon stability at pH2.

Each sample was assayed for IF activity before and after the treatment at pH2. The pH of the supernatants, containing IF, was lowered using 1NHCl to pH2 and stored at 4^{o}C for 24 hours. Following day, the pH was raised back to seven with 1N NaOH. This treatment destroyed the immune IF and the residual activity of the sample was due to virus interferon only.

F. Calculation of IF Titers

The titer was expressed as the $\log_{10}$ of the highest IF dilution at which 50% plaque reduction occurred. It was calculated using the following formula:

$$\text{titer} = \log_{10} \text{IF} = \frac{c/2 - X_1}{X_2 - X_1} (\log_{10} Y_2 - \log_{10} Y_1) + \log_{10} Y_1$$

where:

- c - number of plaques of VSV control
- X_1 - number of plaques of test sample dilution which will give a value lower than a half of the value for VSV control
- X_2 - number of plaques of test sample dilution that will give a value higher than a half of the value for VSV control
- Y_1 - dilution of X_1
- Y_2 - dilution of X_2

All samples were tested twice. Laboratory standard IF control was included in each assay; and the assay with the higher IF control reading was selected. Furthermore, titers of interferon samples were expressed as a fraction. The numerator was the titer of the test IF sample and the denominator was the titer of the standard laboratory IF control, included in the particular assay. The laboratory standard had a stable titer when several samples of it were tested simultaneously. But there was some variation present in the titer of the IF control when assayed at different times. Therefore, to eliminate this variability,

attributed to cell cultures rather than the IF control itself, the titers of the test interferon samples were compared with respect to that of the standard laboratory IF control.

The interferon produced in vitro in supernatants of lymphocyte cultures, before the treatment at pH2, is referred to as the "total" IF. It is subdivided into the "viral" and "immune" IF according to the stability at pH2 for 24 hours. The "viral" IF, being acid stable, was the IF titer still present after the 24 hour treatment at pH2; whereas the "immune" IF, being acid labile, was the IF titer lost after the exposure at pH2, which numerically equalled to the difference between the "total" IF and the "viral" IF. A change in the titer, after the exposure to pH2, of less than one $\log_{10}$ was not considered significant.

V. Serological Studies

These tests were kindly performed by the Cadham Laboratories in Winnipeg. Rubeola antibodies were determined by microtiter hemagglutination inhibition (Lennette and Schmidt 1970). Rubeola titers were expressed as reciprocals of serum concentrations.

The separation of immunoglobulins by density gradient centrifugation was performed by the method of Heffner and Schluederberg (1967). Part of the serum was layered on a linear sucrose gradient (12% - 35% weight per volume) and centrifuged for 16 hours at 35,000 rpm using

SW-39 rotor. To obtain IgM antibodies the bottom one-third of the gradient was collected. This portion contained the bulk of 19S activity and was not contaminated with IgG antibodies. The fraction was then examined for measles antibody.

R E S U L T S

A. LYMPHOCYTE TRANSFORMATION AND ANTIBODY TITERS

Group 1. Seronegative Adults Undergoing Immunization

Nine seronegative (rubeola hemmagglutination inhibition titer less than 10) adults between 19 and 36 years of age had no detectable lymphocyte response ($SI \leq 1$) to rubeola antigen before and three to seven weeks after vaccination with live attenuated measles vaccine (Table 2). One adult out of three tested at first week and one adult out of four tested at second week following vaccination responded to rubeola antigen with stimulation indices of 2.8 and 5.1 respectively. The latter adult experienced atypical measles syndrome. She was febrile, had pulmonary infiltrates and developed papular rash ten days after vaccination. She showed seroconversion to measles antigen. One other adult seroconverted from <5 to 10. The highest rubeola antibody titer otherwise was 10 four to seven weeks after vaccination.

No difference was found between the lymphocyte responses to PHA before or after immunization.

Group II. Seronegative Canadian Indian Children Undergoing Immunization

Seven Canadian Indian children between the ages of one and nine were studied. Two of them showed an increase in lymphocyte response (stimulation index had at least doubled) to rubeola antigen four weeks following vaccination

with live attenuated measles vaccine (Table 3). The mean of the stimulation indices to rubeola antigen four weeks after vaccination was significantly higher (Student's t test; $\alpha > 0.05$) than before vaccination. There was no difference found in the lymphocyte responses of these children to PHA before (mean 21 ± 15) and after vaccination (29 ± 7) with live attenuated measles vaccine. All children in this group showed seroconversion to rubeola antigen following vaccination with titers ranging from 20 to 160. Two of them also had a IgM antibody to measles.

Group III. Children convalescing from an episode of atypical measles.

These two children experienced atypical measles four weeks prior to testing. Both were febrile, had pulmonary infiltrates and developed papular rash. They received killed measles vaccine when they were three and four years old. Both of them showed highest lymphocyte response out of all donors tested to rubeola antigen four weeks following their illness, with stimulation indices of 8.7 and 5.2 (Table 4).* One child showed a decreased response to PHA. Their antibody titers to rubeola were 16 and less than 4 respectively at the time of the onset of rash while four weeks later, they both showed antibody titers to rubeola of 160.

*For comparison of these responses see table 3, under the heading "before vaccination".

Group IV. Adults undergoing reexposure to measles virus.

Four adults between the ages of 24 and 37, thought to be already immune to measles were immunized with measles virus vaccine. Two of these adults were seropositive (rubeola hemagglutination inhibition titer of 10 or greater) with a childhood history of natural measles. The other two were seronegative (rubeola antibody titer less than 10) in spite of being previously vaccinated with live attenuated measles vaccine within the past seven months. None of the adults showed lymphocyte response to rubeola antigen before or after revaccination (SI <1) with measles vaccine. There was no difference in the stimulation indices to PHA before (mean 16±10) or after (21±13) revaccination (Table 5). Antibody titers did not change for up to four weeks following reexposure to measles virus. The seropositive donors had no detectable IgM antibody, even after immunization.

Group V. Control seropositive adults

This group of adults, 22 to 37 years of age, had experienced natural measles in childhood. Their antibody titers ranged from 20 to 160. None of the donors exhibited a lymphocyte response to rubeola antigen; but they all showed normal lymphocyte response to PHA (Table 6).

B. INTERFERON PRODUCTION

Group I. Seronegative adults undergoing immunization

The production of IF at various time intervals before and after the vaccination of nine donors was studied. The IF titers produced in cultures stimulated with rubeola antigen are shown in Table 7. The peak of IF production occurred two weeks after immunization. The mean titer of "immune" IF for donors 2,3,6 and 8 was significantly higher (Student's t test; $\alpha > 0.5$) two weeks following vaccination (1.4 ± 0.4) than before vaccination (0.25 ± 0.03). No differences were found in the IF production before vaccination, at 1,3,4, and 5-7 weeks after vaccination.

The IF titers produced as a result of PHA stimulation are presented in Table 8. The peak of "immune" IF production occurred three weeks after immunization with live attenuated measles vaccine. None of the donors tested produced "immune" IF before and one week after the vaccination.

Group II. Canadian Indian children with negative measles history undergoing immunization

The IF titers produced as a result of rubeola antigen stimulation are summarized in Table 9. There was no significant "immune" IF production before and four weeks after (titer less than one) the vaccination with live attenuated measles vaccine. Unfortunately, we were not able to obtain samples at 2 weeks after immunization.

The IF production as a result of PHA stimulation is shown in Table 10. No statistical difference (Student's t test; $\alpha > 0.05$) was found in the amount of IF produced before or after the vaccination. Two children produced "immune" IF before vaccination but not after; however, the mean of "immune" IF production was not specifically different before (0.8 ± 0.8) and after (0.5 ± 0.1) the vaccination.

Group III. Children convalescing from an episode of atypical measles.

Both donors produced "immune" IF on stimulation by rubeola antigen four weeks following atypical measles (Table 11).* One donor produced "immune" IF to PHA at that time.

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*For comparison of these results see table 9 and 10 under the column heading "before vaccination".

Group IV. Adults undergoing reexposure to measles virus.

IF production after a second exposure to measles virus was studied in four adult donors. The peak of IF response as a result of rubeola antigen stimulation occurred two weeks after the vaccination with attenuated measles vaccine (Table 12). However, the changes were not statistically significant.

In the donors' cultures incubated with PHA, the peak of the "immune" IF production was observed two weeks following vaccination.

Group V. Control seropositive adults

This control group had no significant production of "immune" IF in cultures stimulated with rubeola antigen (titer less than one).

The IF titers of lymphocyte cultures incubated with PHA were less than one (Table 13).

C. CONTROL ANTIGENS

i. Rubella antigen: The stimulation indices of the lymphocyte responses to rubella antigen ranged from 0.3 to 3.0. The sample mean before vaccination was 1.1±.7 and after vaccination was 1.4±0.5 of all donors studied. Seventy-eight percent of these donors did not produce interferon as a result of rubella stimulation. The other 22% had detectable interferon but there was no difference found between the results at the beginning of the study (mean 1.5±0.4) and at the end (mean 1.3±0.8).

ii. Rubeola control-antigen: The numerical values of the lymphocyte response to rubeola control-antigen ranged from 0.3 to 1.8. There was no change in the lymphocyte response to rubeola control-antigen before (mean 0.8±0.4) and after (mean 0.8±0.3) vaccination of the donors. These results also show that the rubeola control-antigen produced toxicity in lymphocyte cultures since the stimulation indices were consistently lower than unity with respect to the unstimulated lymphocyte cultures. Interferon production was not detected as a result of rubeola control-antigen stimulation.

Table 2. Stimulation indices of nine seronegative* adults with unknown measles history undergoing immunization with live attenuated measles vaccine.

DONORS	AGE	BEFORE VACCINATION RUBEOLA Ab** TITER	PHA***	RUBEOLA+	FIRST FOLLOW UP (1-3 WEEKS) PHA	RUBEOLA	SECOND FOLLOW UP (4-7 WEEKS) PHA	RUBEOLA	RUBEOLA Ab TITER AT END OF STUDY
1	19	5	NS++	.85	57	2.8	42	1.4	10
x 2	31	< 5	12	.97	34	5.1	4.3	.92	80
3	40	< 5	NS	1.0	28	1.0	10	.45	10
4	30	< 5	NS	1.4	14	1.2	67	1.0	< 5
5	20	< 5	5.1	.55	16	.77	7.1	.71	< 5
6	22	5	15	.92	37	.96	NS	NS	10
7	27	5	22	.52	17	.56	14	.49	10
8	28	5	NS	1.1	6.8	1.1	6.6	.46	< 5
9	36	< 5	130	1.4	7.3	.89	3.9	.27	< 5
MEAN± SD+++	28±7		37±52	1.0±.3	24±16	1.6±1.5	19±23	.7±.4	

* rubeola antibody titer of less than 10
** antibody
*** Phytohemagglutinin stimulation
\+ stimulation with Rubeola antigen
++ no sample
+++ standard deviation
x atypical measles

Table 3. Stimulation indices of seven Canadian Indian children with negative measles history undergoing immunization with live attenuated measles vaccine.

DONOR INITIALS	AGE IN YEARS	BEFORE VACCINATION		4 WEEKS FOLLOWUP		RUBEOLA Ab+ TITER AT END OF STUDY	IgM++
		PHA*	RUBEOLA**	PHA	RUBEOLA		
B.B.	1	10	1.0	36	1.9	160	Negative
K.C.	5	40	1.0	39	1.5	160	Positive
K.R.	3	17	1.8	21	2.2	160	Negative
S.R.	6	18	1.0	26	1.4	20	NS+++
V.R.	9	45	1.0	34	1.4	80	Negative
T.S.	1	11	1.0	25	3.6	160	Positive
S.S.	2	7.8	1.1	21	2.3	160	Negative
Mean ±SD	4±3	21±15	1.1±0.3	29±7	2.0±0.8	$10x2^{3.43}$	

* stimulation with Phytohemagglutinin
** stimulation with rubeola antigen
\+ antibody
++ immunoglobulin M
+++ no sample

Table 4. Stimulation indices of two children recently convalescent from atypical measles.

DONOR INITIALS	AGE IN YEARS	RUBEOLA Ab* TITER DURING RASH	PHA**	FOUR WEEKS FOLLOW UP	
				RUBEOLA***	RUBEOLA Ab TITER
B.S.	12	16	26	8.7	160
C.S.	13	< 4	3.2	5.2	160
MEAN±SD	12.5±0.5		15±16	7±2	$10x2^4$

* antibody
** stimulation with Phytohemagglutinin
*** stimulation with rubeola antigen

Table 5. Stimulation indices of four adults thought to be already immune to measles were immunized with live attenuated measles vaccine. Two adults have a childhood history of measles, the other two have been vaccinated with live attenuated measles vaccine within the past seven months without a rise in antibody titer.

DONORS	AGE IN YEARS	BEFORE REVACCINATION			2 WEEKS FOLLOW UP		4 WEEKS FOLLOW UP		
		RUBEOLA Ab* TITER	PHA**	RUBEOLA***	PHA	RUBEOLA	RUBEOLA Ab TITER	PHA	RUBEOLA
10	24	40	18	1.3	13	.73	40	12	.87
11	37	40	7.3	.73	37	1.4	40	58	.95
12	30	< 5	29	1.0	27	.98	5	45	1.1
13	36	< 5	10	.27	8.1	.48	5	4.6	.41
MEAN±SD+	32±6		16±10	.8±.4	21±13	.9±.4		30±26	.83±.3

* antibody
** stimulation with Phytohemagglutinin
*** stimulation with rubeola antigen
\+ standard deviation

Table 6. Stimulation indices of seven control adults with a childhood history of measles.

DONORS	AGE IN YEARS	RUBEOLA Ab* TITER	PHA**	RUBEOLA***
14	24	40	10	1.3
15	29	20	10	.76
16	29	40	16	1.0
17	23	160	23	.63
18	37	40	7.3	.73
19	22	40	15	1.6
20	29	160	5.4	1.2
MEAN±SD+	28±5	$10x2^{2.43}$	12±6	1.0±.4

* antibody
** stimulation with Phytohemagglutinin
*** stimulation with rubeola antigen
\+ standard deviation

Table 7. Interferon titers of nine adult donors before and up to seven weeks after vaccination with live attenuated measles vaccine. The interferon was produced *in vitro* by lymphocyte cultures of the donors as a result of rubeola antigen stimulation.

	BEFORE VACCINATION			1 WEEK FOLLOW UP			2 WEEKS FOLLOW UP			3 WEEKS FOLLOW UP			4 WEEKS FOLLOW UP			5-7 WEEKS FOLLOW UP		
DONORS	TOTAL IF[+]	VIRAL IF[++]	IMMUNE IF[+++]	TOTAL IF	VIRAL IF	IMMUNE IF	TOTAL IF	VIRAL IF	IMMUNE IF	TOTAL IF	VIRAL IF	IMMUNE IF	TOTAL IF	VIRAL IF	IMMUNE IF	TOTAL IF	VIRAL IF	IMMUNE IF
1	1.0	1.0	.0	2.4	1.4	1.0										1.0	1.0	0
2 *	1.9	1.5	0.4				3.3	1.5	1.8				2.5	2.0	0.5			
3	1.0	1.0	0				3.1	1.5	1.6							2.3	1.0	1.3
4	1.0	1.0	0	1.4	1.1	0.3							1.0	1.0	0			
5	2.0	1.6	0.4							2.0	1.0	1.0				1.8	1.8	0
6	1.8	1.5	0.3				2.6	1.0	1.6									
7	1.2	1.2	0							2.0	2.1	-0.1	1.9	1.3	0.6			
8	2.0	1.7	0.3				2.2	1.8	0.4							2.4	2.0	0.4
9	2.0	1.8	0.2	1.6	1.0	0.6							1.0	1.0	0			
MEAN±SD	1.5± 0.5	1.4± 0.3	.2±.2	1.8± .5	1.2± 0.2	.6± .4	2.8± .5	1.5± .3	1.4± .3	2±0	1.6± .8		1.6± .7	1.3± .5	.3± .3	1.9± .6	1.5± .5	.4± .6

\+ Total Interferon - the titer of IF produced in supernatants of lymphocyte cultures.
++ Viral Interferon - the titer of IF left after 24 hour treatment at pH2 (acid stable).
+++ Immune Interferon - the titer of IF lost after the exposure at pH2 (acid labile).
* Atypical Measles

Table 8. Interferon titers of nine adult donors before and up to seven weeks following vaccination with live attenuated measles vaccine. The interferon was produced *in vitro* by lymphocyte cultures of the donors as a result of PHA stimulation.

	BEFORE VACCINATION			1 WEEK FOLLOW UP			2 WEEKS FOLLOW UP			3 WEEKS FOLLOW UP			4 WEEKS FOLLOW UP			5-7 WEEKS FOLLOW UP		
DONORS	TOTAL[+] IF	VIRAL[++] IF	IMMUNE[+++] IF	TOTAL IF	VIRAL IF	IMMUNE IF	TOTAL IF	VIRAL IF	IMMUNE IF	TOTAL IF	VIRAL IF	IMMUNE IF	TOTAL IF	VIRAL IF	IMMUNE IF	TOTAL IF	VIRAL IF	IMMUNE IF
1				1.0	1.0	0										1.0	1.0	0
2 *	1.0	1.0	0				4.1	2.2	1.9				2.3	1.7	0.6			
3							1.0	1.0	0							1.5	1.0	0.5
4				1.0	1.0	0							1.0	1.0	0			
5										2.0	1.0	1.0				0.9	1.0	-0.1
6	1.0	1.0	0				3.2	1.0	2.0									
7	1.0	1.0	0							3.2	1.5	1.7	2.8	1.0	1.8			
8	1.0	1.0	0				1.0	1.0	0							2.8	1.0	1.8
9	1.5	1.5	0	1.0	1.0	0							1.0	1.0	0			
MEAN± SD	1.1± .2	1.1± .2	0	1±0	1±0	0	2.3± 1.6	1.3± .6	1.0± 1	2.6 ±.8	1.3± .4	1.4± .5	1.8± ±.9	1.2± .4	.6± .8	1.6± .9	1±0	.6±.8

\+ Total Interferon - the titer of IF produced in supernatants of lymphocyte cultures.
++ Viral Interferon - the titer of IF left after 24 hours treatment at pH2 (acid stable).
+++ Immune Interferon - the titer of IF lost after the exposure at pH2 (acid labile).
* Atypical Measles

Table 9. Interferon titers of seven seronegative Canadian Indian children before and four weeks after immunization with live attenuated measles vaccine. The interferon was produced in vitro by lymphocyte cultures of the donors as a result of rubeola antigen stimulation.

DONORS	BEFORE VACCINATION			4 WEEKS FOLLOW UP		
	TOTAL+	VIRAL++	IMMUNE+++	TOTAL	VIRAL	IMMUNE
BB	1.5	1.0	0.5	2.5	NS	NS
KC	1.6	1.3	0.3	1.8	1.8	0
KR	1.6	NS*	NS	1.5	1.5	0
SR	1.0	1.0	0	1.0	1.0	0
VR	1.8	1.5	0.3	1.5	1.5	0
TS	1.5	1.3	0.2	1.4	1.0	0.4
SS	2.3	1.5	0.8	1.5	1.5	0
MEAN±SD	1.6±.4	1.3±.2	.4±.3	1.6±.5	1.4±.3	.1±.2

\+ **Total** Interferon - the titer of IF produced in supernatants of lymphocyte cultures.
++ Viral Interferon - the titer of IF left after 24 hour treatment at pH2.
+++ Immune Interferon - the titer of IF lost after the exposure at pH2.
* No sample

Table 10. Interferon titers of seven seronegative Canadian Indian children before and four weeks after immunization with live attenuated measles vaccine. The interferon was produced in vitro as a result of PHA stimulation.

DONORS	BEFORE VACCINATION TOTAL+ IF	VIRAL ++ IF	IMMUNE+++ IF	4 WEEKS FOLLOW UP TOTAL IF	VIRAL IF	IMMUNE IF
BB	1.8	1.0	0.8	3.2	NS*	NS
KC	1.2	1.0	0.2	1.3	1.0	0.3
KR	3.3	1.5	1.8	1.5	1.0	0.5
SR	3.3	1.5	1.8	1.7	1.0	0.7
VR	2.3	1.5	0.8	2.0	1.5	0.5
TS	1.0	1.0	0	1.5	1.0	0.5
SS	1.5	1.5	0	1.5	1.0	0.5
MEAN±SD	2.1±1.0	1.3±0.3	0.8±0.8	1.8±0.7	1.1±0.2	0.5±0.1

\+ Total Interferon - the titer of IF produced in supernatants of lymphocyte cultures.
++ Viral Interferon - the titer of IF left after 24 hour treatment at pH2.
+++ Immune Interferon - the titer of IF lost after the exposure at pH2.
* No sample.

Table 11. Interferon titers of two children convalescing from a recent episode of atypical measles. The interferon was produced in vitro by lymphocyte cultures as a result of PHA and rubeola antigen stimulation.

DONOR STUDY NO.	TOTAL+ IF		VIRAL++ IF		IMMUNE+++ IF	
	PHA	RUBEOLA	PHA	RUBEOLA	PHA	RUBEOLA
30	1.6	1.6	0.6	0.6	1.0	1.0
29	2.0	2.0	1.1	1.0	0.9	1.0
MEAN±SD	1.8±.3	1.8±.3	.9±.4	.8±.3	1±.1	1.0±0

\+ Total Interferon - the titer of IF produced in supernatants of lymphocyte cultures

++ Viral Interferon - the titer of IF left after 24 hour treatment at pH2

+++ Immune Interferon - the titer of IF lost after the exposure at pH2

Table 12. Interferon titers of four adult donors before and up to four weeks after vaccination with live attenuated measles vaccine. The first two of the above donors had a rubeola titer of 40 with a childhood history of natural measles. The other two donors had a rubeola titer of less than 5 and they have had live attenuated measles vaccine within past seven months. The interferon was produced in vitro by lymphocyte cultures of the donors as a result of PHA and rubeola antigen stimulation.

A. PHA STIMULATION

DONORS	BEFORE REVACCINATION			2 WEEKS FOLLOW UP			4 WEEKS FOLLOW UP		
	TOTAL+ IF	VIRAL++ IF	IMMUNE+++ IF	TOTAL IF	VIRAL IF	IMMUNE IF	TOTAL IF	VIRAL IF	IMMUNE IF
10	1.4	1.0	0.4	2.3	1.3	1.0	1.6	1.3	0.3
11	1.0	1.0	0	1.5	1.0	0.5	1.5	1.3	0.2
12	1.0	1.0	0	1.6	1.5	0.1	1.6	1.3	0.3
13	1.3	1.0	0.3	2.6	1.3	1.3	1.7	1.6	0.1
MEAN±SD	1.2±0.2	1±0	.2±.2	2±.5	1.3±.2	.7±.5	1.6±.5	1.4±.2	.2±.1

B. RUBEOLA ANTIGEN STIMULATION

10	1.8	0.8	1.0	2.6	1.2	1.4	1.5	1.2	0.3
11	1.3	0.8	0.5	1.8	1.1	0.7	1.2	1.2	0
12	1.6	0.8	0.8	1.8	1.3	0.5	1.2	1.2	0
13	1.8	1.4	0.4	2.5	1.5	1.0	1.6	1.4	0.2
MEAN±SD	1.6±.2	1.0±.3	.7±.3	2.2±.4	1.3±.2	0.9±.4	1.4±.2	1.3±.1	.1±.1

\+ Total Interferon - the titer of IF produced in supernatants of lymphocyte cultures.
++ Viral Interferon - the titer of IF left after 24 hour treatment at pH2.
+++ Immune Interferon - the titer of IF lost after the exposure at pH2.

Table 13. Interferon titers of seven control adult donors with a history of natural measles in childhood and a rubeola antibody titer ranging from 20 to 160. The interferon was produced *in vitro* by lymphocyte cultures of the donors as a result of rubeola antigen stimulation.

DONORS	TOTAL+ IF	VIRAL++ IF	IMMUNE+++ IF
14	1.8	0.8	1.0
15	1.9	1.5	0.4
16	1.0	1.0	0
17	2.0	1.6	0.4
18	1.3	1.0	0.3
19	1.0	1.0	0
20	1.8	1.8	0
MEAN±SD	1.5±.4	1.2±.4	.3±.4

+ Total Interferon - the titer of IF produced in supernatants of lymphocyte cultures

++ Viral Interferon - the titer of IF left after 24 hour treatment at pH2.

+++ Immune Interferon - the titer of IF lost after the exposure at pH2.

D I S C U S S I O N

Antibody response following measles infection

Following the immunization with measles vaccine "Attenuvax - more attenuated Enders' line of Edmonton Strain" by Merck, Sharp and Dohme, all of the 7 Indian children developed measles antibodies. Their geometric mean titer (GMT) was 108 four weeks after vaccination. Two children who experienced atypical measles four weeks prior to testing also developed antibodies with a GMT of 160.

On the other hand, all but one of nine donors failed to develop increased antibody titers. The highest rubeola antibody titer was 10 up to seven weeks following vaccination. The only exception was donor #2 who developed atypical measles syndrome ten days after vaccination. Her antibody titer rose from less than 5 to 80.

Atypical illness on exposure to wild measles virus had been observed in children previously immunized with inactivated measles vaccine, as well as in children who received one dose of live vaccine shortly after receiving killed vaccines (Fulginiti et al, 1967; Norrby et al, 1975). A case of atypical measles was also reported in a twelve years old patient who received live measles vaccine several years after receiving a

killed vaccine. Such atypical illness could have been influenced by the following factors. First, the live attenuated virus was destroyed by antibodies created by the prior immunization with killed vaccine. Secondly, the live measles vaccine was not live at that particular instance. Thirdly, the live vaccine itself contributed to altered reactivity of the patient (Chatterji and Mankad, 1977).

In our study we had two children with atypical illness who received killed measles vaccine, and one adult with atypical illness with no history of receiving killed vaccine. This adult was also said to have had a childhood history of measles without detectable measles antibodies prior to vaccination. A similar case of severe, unusual, exanthematous illness associated with a rise in measles HAI antibody titer was reported in a child who had previous measles plus two measles vaccinations with attenuated vaccine (St. Geme et al, 1976).

Atypical measles syndrome is not a simple vaccine failure. The nature of the disease implies that the host responsiveness to wild measles virus is altered. Fulginiti and his co-workers suggested that delayed hypersensitivity may be induced by inactivated measles virus vaccine (Fulginiti et al, 1967). Moreover, it was also suggested that on rare occasions the immunologic imbalance, between the persistent CMI in the absence of once-present humoral antibody, might result from repeated inoculations of live, attenuated measles virus vaccine

(St. Geme et al, 1976). Although immunologic process has been implied in atypical measles, the exact mechanism is still obscure.

Significantly, however, there was no change observed in antibody titers following reimmunization of two adults with past history of measles and rubeola antibody titer of 40. After the revaccination of another two adults with initial rubeola titer of less than 5, there was also no rise in rubeola antibody titer in the following four weeks. As a child one of these seronegative adults had an exposure to a wild measles virus during a measles outbreak. Nevertheless, while her siblings contracted measles, she did not. Thus, despite the fact that she has no antibody titer to measles at present, most likely she has a cellular immunity to measles. Similar results were reported by another group of investigators (Ruckdeschel et al, 1975). However, they were able to demonstrate the cellular immunity in two seronegative pediatric residents who were frequently exposed to measles.

Our results are also similar to those of Deseda-Tous and her co-workers (1978) who studied the antibody response in 26 children with initial rubeola antibody titers of less than 5. The GMT of those with a primary response was 90 and 40, three weeks and ten months after vaccination respectively. In contrast, the children with secondary immune response had GMT of 28 and 9 three weeks and ten months after vaccination. They suggested, therefore that a "booster" reimmunization with the

presently available measles vaccine might not be effective in stimulating sustained antibody in those persons previously antigenically stimulated by measles virus.

Lymphocyte Transformation

CMI measured by H^3 thymidine incorporation was detected following atypical measles of two children and an adult donor. The stimulation indices were 8.7,5.2, and 5.0 respectively. Seven Canadian Indian children developed a significantly higher mean ($\alpha = 0.05$) of stimulation indices four weeks after vaccination (2.0 ± 0.8) than before (1.1 ± 0.3). These results suggest that the magnitude of CMI response following vaccination is less than that following atypical measles syndrome.

We were not able to demonstrate lymphocyte response to rubeola in the seropositive adult group with past history of measles. This was probably due to assay sensitivity which might have been affected by the serum and the rubeola antigen used in lymphocyte cultures. Haahr and his co-workers (1976) had observed in their study of herpes simples virus (HSV) that the use of human AB serum which had no antibodies to HSV tended to increase the lymphocyte response as compared to the response of cultures grown in the presence of fetal calf serum. The AB serum applied to our system could probably improve the results of the seropositive adult group with measles in the remote past.

The quality of antigen used for lymphocyte stimulation is a very important parameter. The commercially prepared rubeola complement fixation antigen involved in this assay had been used successfully by other investigators (Ruckdeschel et al, 1975, Graziano et al, 1975; Kreeftenberg and Loggen 1977). However, Kreeftenberg and Loggen reported that a number of lots of measles complement fixation antigen they used did not stimulate lymphocytes at all. This might explain some of our results.

It had also been noted in our study that the rubeola control-antigen produced certain amounts of toxicity in lymphocyte cultures. The transformation index for the cultures incubated with rubeola control-antigen was consistently lower than unity as compared to cultures incubated only with growth media. Similar inhibition was reported by Sullivan and his colleagues. Both the unpurified measles virus and the control fluid from uninfected vero cell cultures were found to be inhibitory when compared to medium RPMI 1640. They also observed that partial purification of measles virus preparations eliminated the inhibitory effect caused by cell-derived factors (Sullivan et al, 1975). In addition, other investigators compared crude and dextran 10-purified HSV antigens as cell culture stimulants. They demonstrated that not only a better reproducibility was achieved but also an almost doubling of H^3 thymidine incorporation was observed when a purified HSV antigen was employed (Haahr et al, 1976). Therefore, a laboratory prepared and purified antigen rather

than commercially obtained one might be a better choice of measles antigen in order to increase the overall assay sensitivity.

Interferon Production

Because of the different procedures investigators used to study interferon production, it is difficult to compare their results. Using HSV antigen as an inducer of interferon, Haahr and his associates concluded that interferon produced in ficoll-hypaque purified mononuclear cultures was type I and was unrelated to the immune status. Type II or immune interferon occurred in either macrophage-lymphocyte or column purified lymphocytes cultures (Haahr et al, 1976).

On the other hand, we were able to detect immune interferon following measles infection in ficoll-isopaque purified mononuclear cell cultures. This result was consistent with observations made by Buimovici-Klein and her co-workers (1977), that macrophage-lymphocyte cultures were not a requirement for the production of immune interferon following rubella antigen stimulation. Furthermore, another group of researchers observed that when a more heterogeneous cell population, as in ficoll-hypaque cultures, was stimulated with HSV antigen, what appeared to be either a third type of interferon, was produced (Valle et al, 1975b). These results seem to suggest that although macrophage-lymphocyte cultures may be better with respect to the purity

of immune interferon obtained, immune interferon can nevertheless be produced in ficoll-hypaque cultures. The latter method of lymphoid cell preparation is more practical than the former one, since only half the amount of blood is required and the test can be completed in one visit. This is especially favorable when working with children.

Even though type I interferon was produced by all donors tested, significant immune interferon production with rubeola antigen was limited to the seronegative adults tested at two weeks after vaccination and the group of children with recent experience of atypical measles. We also observed an increase in the immune interferon production following revaccination of the adults; however, the amount produced was not significant. No interferon was produced with rubeola control antigen. Furthermore, there was either no interferon produced or no increase in interferon production with rubella antigen following measles vaccination. These findings suggested that the immune interferon was produced specifically as a result of rebeola stimulation.

Why seronegative children did not produce immune interferon following immunization is not quite clear. The peak of immune interferon production in adults occurred two weeks after the vaccination; and by four weeks it declined to insignificant levels. Since we were not able to obtain samples at two weeks, the children were tested only at four weeks after vaccination, hence we should not exclude

the possibility that we might have missed the time when immune interferon was produced.

We were able to detect immune interferon production following PHA stimulation in cultures of children, after recent episode of atypical measles, and of seronegative adults, two to seven weeks after measles vaccination. However, this group of seronegative adults did not produce interferon before immunization as well as one week afterwards. Depression of lymphocyte responsiveness following measles infection could perhaps explain the lack of immune interferon production one week after the immunization.

Phytohemagglutinin and purified protein derivative are known to stimulate type II interferon in mice (Youngner and Salvin, 1973). Valle and associates also reported that both human macrophage-lymphocyte cultures and ficoll-hypaque cultures produced immune interferon as a result of PHA stimulation (Valle et al, 1975b). This might well be due to the fact that PHA is a potent T cell stimulator in man. However, we observed type I or viral interferon produced following PHA stimulation. One possible explanation might be that this interferon was produced as a result of stimulation by nonspecific cell-derived factors in cultures.

It is interesting to note that of all adults tested, the donor who developed atypical measles syndrome showed the highest immune interferon production to rubeola

antigen following the vaccination. Similarly, of all children tested, the two children with atypical measles also produced the highest level of immune interferon to rubeola and PHA stimulation. Our results suggest that a higher immune response is produced following atypical measles than following vaccination.

Furthermore, our results seem to suggest that there are two groups of seronegative adults: those who are resistant to measles infection and those who are susceptible. The first group has no antibodies to measles but they have cellular immunity (either by lymphocyte responsiveness or immune interferon production), which they probably acquired from the exposure to wild measles or other related viruses (eg. canine distemper virus). They do not produce antibodies even after re-vaccination. The second group of seronegative adults also has no cellular immunity because they have not been exposed to measles or related viruses.

SUMMARY

The results are listed in table 14.

1. Positive results in antibody production, lymphocyte response and immune interferon production were obtained only following atypical measles.
2. Atypical measles produced higher response than vaccine infection.
3. Vaccination of seropositive and seronegative adults had no effect on either humoral or cell-mediated immune responses.
4. Following vaccination of seronegative adults and Canadian Indian children cell-mediated immunity was detected by either lymphocyte response or immune interferon production but it was not detected simultaneously by both methods.
5. Live attenuated measles vaccine was not successful in stimulating humoral immunity of nine seronegative adults, with one exception.

Table 14. Comparison of methods used for detection of measles immunity.

Group	Measles antibody	Transformation index	Immune interferon
9 seronegative adults following vaccination (except donor #2)	no rise	1/8* positive	significant increase
7 seronegative children following vaccination	rise GMT** 108	significant increase	negative
2 children + donor #2 after atypical measles	rise GMT 127	3/3 positive	3/3 positive
4 vaccinated adults thought to be already immune	no change	negative	negative
7 seropositive adults with past history of measles	GMT 54	negative	negative

* number of positives/number tested
** geometric mean titer

B I B L I O G R A P H Y

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