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Interferon-Induced 2-5A Synthetase Activity in Human Peripheral Blood Mononuclear Cells After Immunization with Influenza Virus and Rubella Virus Vaccines

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The interferon-induced enzyme 2-5A synthetase can be a sensitive indicator of activation of the human interferon system during viral infection or interferon therapy. To determine the response of the human interferon system to viral antigens, the level of 2-5A synthetase activity was monitored in peripheral blood mononuclear cells of healthy adults before and after immunization with influenza or rubella virus vaccine. The influenza virus-vaccinated individuals demonstrated increases in enzyme activity on days 1 and 11 in vivo, whereas those vaccinated with rubella virus vaccine showed an increase only on day 11. The difference in the day 1 in vivo 2-5A synthetase response in the two vaccinated groups could be demonstrated by in vitro incubations of peripheral blood mononuclear cells isolated approximately 90 days postvaccination with the two vaccines. The day 11 increase of enzyme activity in the rubella virus group showed a positive correlation with an increase in serum antibody titer, suggesting activation of the interferon system during antibody production in vivo after human exposure to virus antigens. The demonstration of increased 2-5A synthetase activity at specific times postimmunization in this investigation indicates that the interferon system is involved in the human in vivo response to virus vaccination.

Interferons (IFN) are produced by animals or cultured cells in response to a variety of inducers, including viral infection or polynucleotides. The characteristics of the stimulus and the type of cell involved combine to determine which of the three antigenically and physically distinct types of IFN (α, β, or γ) are produced (reviewed in reference 23).

Cells exposed to IFN undergo a large number of biochemical and functional changes. However, the precise contribution of these multiple biological effects and the role of IFN in the recovery from and resistance to viral infection in vivo have been difficult to determine. Administration of neutralizing IFN antibodies to animals has demonstrated the importance of endogenous IFN production in reducing viral replication and host mortality (6). In humans, an in vivo role for IFN in the recovery from viral disease has been implied from its detection in human serum and tissues during acute viral infections. However, direct investigations monitoring IFN throughout a course of human viral disease have been hampered by the irregular availability of blood samples, drug therapy, and the wide fluctuations and rapid disappearance of serum IFN activity. These factors make it difficult to relate the activity of the IFN system to the response of a patient with viral infection.

The mechanism of IFN's potent antiviral action involves the induction of specific cellular proteins to increased levels. One well-characterized IFN-induced protein is 2-5A synthetase. Once activated by double-stranded RNA, this enzyme generates, from ATP, 2'-5'-linked oligoadenylates (2-5A), which in turn activate a latent RNase that cleaves single-stranded RNA at specified sites (14, 24, 28).

Experimental evidence indicates that IFN-induced 2-5A synthetase is involved in mediating IFN's antiviral affect: 2-5A can be detected in IFN-treated, virus-infected cells (25); introduction of 2-5A or 2-5A core (2-5A with 5'-terminal triphosphate removed) into cells results in decreased protein synthesis and virus replication and increased cleavage of RNA (10, 26, 27); and 2-5A synthetase activity is elevated in tissue homogenates, serum, and peripheral blood mononuclear (PBM) cell extracts of mice infected with virus or treated with IFN or IFN inducers (9, 12, 18, 21).

Extracts prepared from PBM cells isolated from patients with viral infections demonstrate elevated levels of 2-5A synthetase activity (20, 29). Patients with elevated serum IFN levels, resulting from viral infection or IFN therapy, also demonstrate elevated 2-5A synthetase activity in PBM cell extracts. Furthermore, although IFN is cleared rapidly from the blood, the activity level of 2-5A synthetase remains elevated in cells for a prolonged period (19, 30). Therefore, it appears that monitoring IFN-induced 2-5A synthetase activity may be a more sensitive and reliable method for detecting IFN activity in vivo than measuring serum IFN levels.

To systematically investigate the human IFN response to viral antigens, IFN and 2-5A synthetase activities were monitored in healthy adults before and after immunization with viral vaccines. This study showed that 2-5A synthetase activity was induced at clearly defined times postimmunization, suggesting that the IFN system is stimulated during the response to viral vaccines. Furthermore, the 2-5A synthetase response of individuals immediately after vaccination in vivo reflects their enzyme response seen in vitro to a particular virus vaccine.

MATERIALS AND METHODS

Virus immunization. Normal healthy human adult volunteers were vaccinated with one of two viral vaccines. Eight individuals (four males, four females) were immunized with 0.5 ml of subvirion, killed, influenza virus vaccine (Connaught Laboratories, Inc., Swiftwater, Pa.). Each dose contained 15 μg of hemmagglutinin of each of the three prototype viral strains A/Brazil/11/78 (H1N1), A/Bangkok/1/79 (H3N2), and B/Singapore/222/79. Individuals seronegative to rubella virus (three males, two females) were immu-

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nized with live, attenuated rubella virus vaccine containing not less than 1,000 50% tissue culture infective doses per 0.5-ml dose (Merck Sharp and Dohme Canada Ltd., Kirkland, Quebec).

Blood samples. Blood samples, 10 to 20 ml with added heparin (Harris Labs, Montreal, Quebec) and 5 ml without heparin, were drawn from each of the vaccinated adults at least 1 day before immunization and twice weekly for approximately 4 weeks after administration of the virus vaccine. Six adult controls (three males, three females) were not vaccinated but did have blood samples drawn by the schedule described above for the immunized adults.

From the heparinized blood, PBM cells were isolated by Ficoll-Hypaque density centrifugation, divided into aliquots (2×10^6 cells per tube), and stored as a cell pellet at −70°C. Cell extracts were prepared by Nonidet P-40 lysis and 2-5A synthetase activity was assayed. The level of enzyme activity measured in PBM cells drawn from an individual and assayed directly is defined as the in vivo determination. The serum was stored at −70°C for a later estimation of the specific virus antibody and serum IFN levels.

To acquire an additional determination of each individual's basal 2-5A synthetase level and to determine the degree of an individual's PBM cell enzyme response after in vitro exposure to IFN, IFN inducers, and the virus vaccines, one blood sample was drawn from each individual approximately 90 days postvaccination. This time interval after immunization was chosen to ensure that the immune system of the vaccinated humans had returned to normal (2). The blood sample was processed as above, and 2×10^6 PBM cells were incubated in vitro with various concentrations of the following: HuIFN-α (Ly) (1.2×10^6 international reference units per ml, 3×10^6 U/mg of protein; Electro-Nucleonics Labs, Inc., Silver Spring, Md.); polyriboinosinic-polyribocytidylic acid [poly(rI) · (rC)] (Sigma Chemical Co., St. Louis, Mo.) or a complex of poly(rI) · (rC), poly-L-lysine, and carboxymethyl cellulose (15); and the virus vaccines as described above. Incubations were also carried out in the presence of hemagglutinin antibody to the three prototype influenza virus strains contained in the influenza virus vaccine (Laboratory Centre for Disease Control, Ottawa, Ontario) or in the presence of HuIFN-α antibody (kindly supplied by Jan Vilcek). These incubations were carried out in vitro for 18 h at 37°C in 5% CO_2 in RPMI 1640 supplemented with 2% human AB serum (Flow Laboratories Rockville, Md.) and 2 mM L-glutamine (GIBCO Laboratories, Grand Island, N.Y.). The samples were centrifuged, and the pellet of PBM cells was stored at −70°C for subsequent assay of 2-5A synthetase activity. The levels of enzyme activity in the PBM cells after the overnight incubations are referred to as the in vitro determinations. The supernatants from these incubations were assayed for the amount and type of interferon produced.

Interferon titrations. Serum samples from vaccinated and nonvaccinated individuals and supernatant from overnight PBM cell incubations were incubated with or without HuIFN-α antibody, or incubated at 56°C for 30 min, or treated overnight at pH 2.0 and then assayed for IFN activity by inhibition of encephalomyocarditis virus cytopathogenic effect in human T98G cells as described previously (4). In this assay, 1 U of National Institutes of Health human reference standard G-023-901-527 was equivalent to 2.8 ± 1.5 laboratory IFN-α standard units.

Assay of 2-5A synthetase activity. Levels of 2-5A synthetase in PBM cell extracts were assayed by using poly(rI) · (rC)-agarose and ion-exchange chromatography as previously described (29, 30). Duplicate PBM cell extracts which were monitored by this protocol and within the same assay demonstrated good reproducibility in results (coefficient of variation, 9%). However, the 2-5A synthetase determined in identical PBM cell extracts by the same assay method but conducted on different days showed an increase in result fluctuations (coefficient of variation, 44%). To ensure that variations in 2-5A synthetase levels were not due to the day-to-day assay variations, all PBM cell samples for each individual were measured within the same assay. The enzyme levels are expressed as picomoles per hour per absorbancy at 260 nm of cell extract.

Virus antibody titrations. The sera of volunteers vaccinated against rubella virus were assayed for specific virus antibody by hemagglutination inhibition before and 6 weeks after immunization (7).

Statistical analysis. To measure the relative variability of 2-5A synthetase activity levels among the vaccinated and control groups over time, the coefficient of variation was calculated and expressed as a percentage (31). This form of measure was also applied to compare the variability of enzyme levels determined within the same assay with those determined in assays conducted on different days.

RESULTS

In vivo 2-5A synthetase activity levels monitored in PBM cells of humans vaccinated with influenza virus. PBM cells were separated from blood samples drawn before and twice weekly for approximately 4 weeks after influenza virus vaccination, and the extracts were assayed for 2-5A synthetase activity. The enzyme activities measured in human PBM cell extracts of each influenza virus-vaccinated individual and four control individuals are shown in Fig. 1. For convenience of illustration, results for only four of six human controls are graphed. The average of the enzyme levels measured when the vaccine would not influence the immune system (before immunization, during weeks 3 and 4 after immunization, and 90 days postimmunization [2]) represents each individual's basal 2-5A synthetase level. Since basal enzyme levels were determined to vary widely among individuals with this assay system, all determinations of 2-5A synthetase activity after vaccination are compared with individual basal enzyme levels. The enzyme levels determined over time in the individuals within the control group fluctuate very little from their respective basal enzyme level (coefficient of variation, 48%). Furthermore, the enzyme fluctuations observed within individuals of this control group occurred in random dates over the time period studied. However, in contrast to controls, six of eight of the influenza virus-vaccinated group demonstrated increased fluctuations in enzyme levels postvaccination (coefficient of variation, 67%), and the changes in enzyme activity occurred at specific times postvaccination. Excluding the two nonresponders (G, H), the pattern of human 2-5A synthetase activity levels in response to influenza virus vaccination includes an initial rise in activity immediately after vaccination, followed by a drop in enzyme activity (days 4 to 8), then an increase in activity (days 10 to 11), and finally a return to basal enzyme level. There did not appear to be any correlation between an individual's enzyme activity and their influenza virus serum antibody titer (data not shown).

As there is good evidence that IFN induces 2-5A synthetase activity in vivo (9, 12, 18, 19, 21), these results suggest that measuring changes in 2-5A synthetase activity can indicate fluctuations in IFN production at specific times after the administration of a virus vaccine.

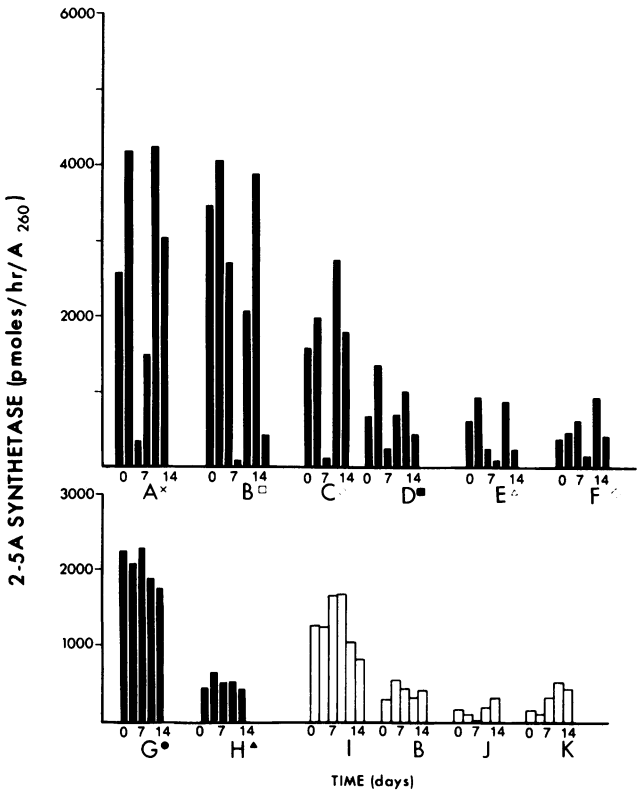

FIG. 1. In vivo time course of 2-5A synthetase levels measured in PBM cells from influenza virus-vaccinated (■) and four of six nonvaccinated control (□) human adults (see text). PBM enzyme levels were determined 1 day before and twice weekly for 4 weeks after the administration of the vaccine. Day 0 represents each individual's basal 2-5A synthetase activity level. The symbols denoting each individual are retained in Fig. 2 and 3. A_{260}, Absorbancy at 260 nm.

In vitro induction of 2-5A synthetase in PBM cells from humans vaccinated with influenza virus. The observed changes in 2-5A synthetase activity levels in vivo after immunization with influenza virus suggests that the vaccination may be stimulating the human IFN system. Therefore, it was of interest to determine whether the 2-5A synthetase levels in PBM cells isolated from the vaccinated group 90 days postvaccination could be elevated by IFN treatment in vitro and whether these enzyme levels reflected the in vivo results. PBM cells were incubated overnight with IFN and the cell extracts were assayed for enzyme activity. The results (Fig. 2) clearly show that treatment of PBM cells with increasing concentrations of IFN increase the levels of 2-5A synthetase activity. Compared with basal enzyme levels, untreated PBM cells incubated overnight demonstrate a decrease in 2-5A synthetase activity. This decrease is not inhibited by cycloheximide treatment (unpublished data) but may result from cell death. The relative increase of enzyme activity in PBM cells induced by IFN is approximately the same among individuals despite their differences in basal enzyme levels.

Treatment of PBM cells from vaccinated volunteers with the IFN inducers poly(rI) · (rC) and poly(rI) · (rC)–poly-L-lysine–carboxymethyl cellulose also resulted in elevated levels of 2-5A synthetase activity (data not shown).

Incubation of PBM cells with the influenza virus vaccine also resulted in enhanced 2-5A synthetase levels measured in the cell extracts in vaccinated (Fig. 3) and control (data not shown) volunteers. IFN activity was detected in the supernatant of these incubations (average, 1,500 U/ml). This IFN activity was neutralized by IFN-α antibody and further characterized as acid and heat stable. The induction of enzyme activity by the influenza vaccine could be inhibited by including IFN-α antibody in the overnight incubation (data not shown). Furthermore, the addition of specific virus hemagglutinin antibody to the in vitro incubations of PBM cells with influenza virus vaccine inhibited the production of IFN and the elevation of 2-5A synthetase activity in these cells (data not shown). This demonstrates that the activation of the IFN system in PBM cells by the influenza virus vaccine was caused by the presentation of virus antigen (hemagglutinin) to the cells. These results suggest that the elevated 2-5A synthetase levels detected in the PBM cells of influenza virus-vaccinated humans 24 h postimmunization result from influenza virus induction of IFN in vivo.

In vivo 2-5A synthetase levels monitored in PBM cells of humans vaccinated with rubella virus. The rubella virus-vaccinated group of volunteers differs from the influenza virus-vaccinated group in two major areas. First, they were administered live, attenuated vaccine, and second, they were immunologically naive with respect to rubella virus. To compare the effect these differences may have on the 2-5A

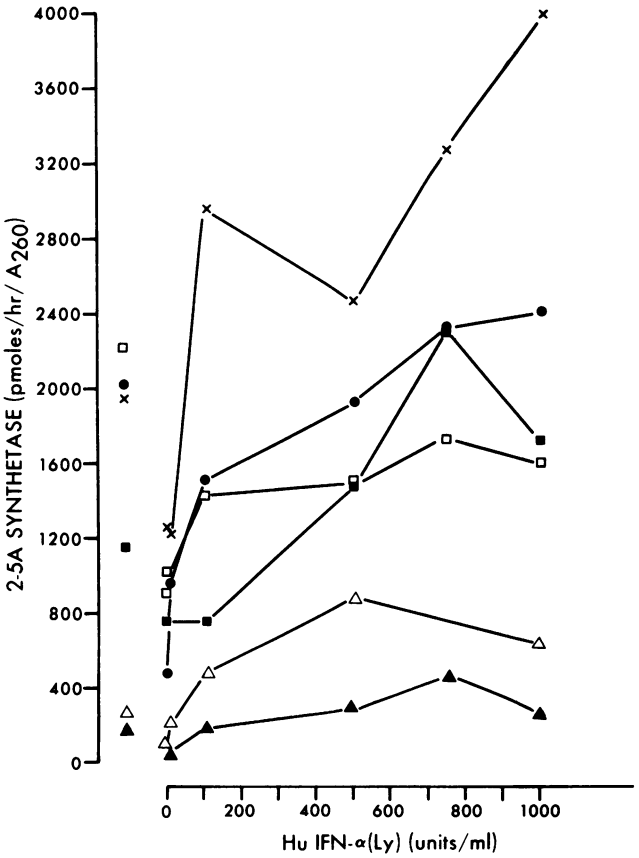

FIG. 2. Effect of HuIFN-α (Ly) on 2-5A synthetase activity levels in human PBM cells isolated 90 days after immunization. Each symbol represents an individual immunized with influenza virus vaccine, and those graphed to the left of the abscissa represent individual basal 2-5A synthetase levels. A_{260}, Absorbancy at 260 nm.

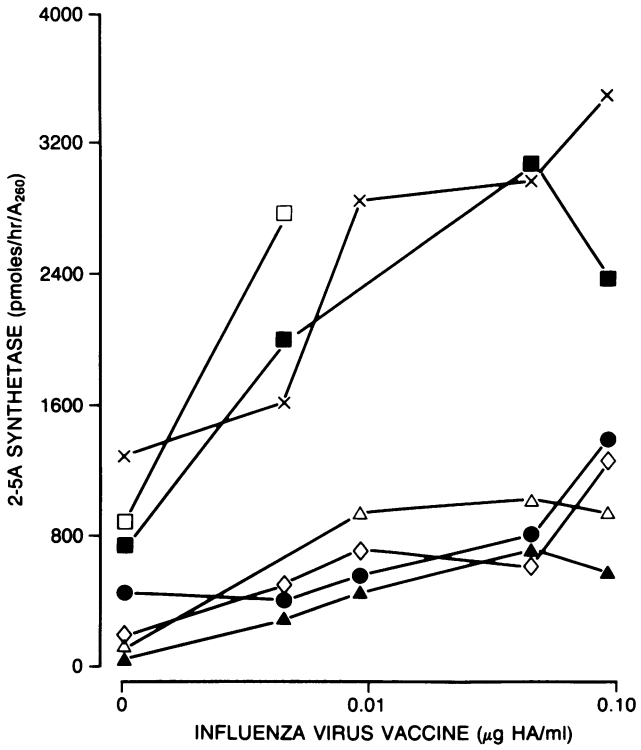

FIG. 3. Response of 2-5A synthetase levels in human PBM cells after overnight incubation with influenza virus vaccine. The PBM cells were isolated from individuals vaccinated with influenza virus approximately 90 days previously. A_{260}, Absorbancy at 260 nm; HA, hemagglutinin.

synthetase response in PBM cells, enzyme levels were monitored before and twice weekly after rubella virus immunization. In contrast to the influenza virus-vaccinated group, the rubella virus-vaccinated group did not demonstrate an increase in enzyme level on day 1 postvaccination (Fig. 4). However, consistent with the influenza virus-vaccinated group, the rubella virus group demonstrated a decrease from basal 2-5A synthetase levels followed by an increase of enzyme activity on day 11 postimmunization. All rubella virus-vaccinated individuals seroconverted to protective titers of serum antibody directed against rubella virus (data not shown).

In vitro induction of 2-5A synthetase in PBM cells of rubella virus-vaccinated individuals. To determine whether the absence of a day 1 increase in 2-5A synthetase in rubella virus-vaccinated individuals was the result of an inability to respond to vaccine induction, PBM cells isolated 90 days postimmunization were incubated overnight with the influenza or rubella virus vaccine and assayed for enzyme activity. The results (Fig. 5) show that, although influenza virus vaccine can induce increasing levels of 2-5A synthetase in these cells, the rubella virus vaccine was inactive in vitro. The treatment of PBM cells from these individuals with IFN or IFN inducers gave results similar to those demonstrated by cells from the influenza virus-vaccinated group.

DISCUSSION

IFN can initiate a variety of biological functions, including the activation of antiviral mechanisms and the modulation of immune responses (23). However, the role of IFN in vivo in the resistance to and recovery from human viral infections remains relatively unclear. Investigations in this area have been hampered by the combined complexities of viral invasion, the human defense response to viral disease, and the biochemistry of the IFN system. The present study has systematically investigated the human IFN response to viral antigens by monitoring IFN-induced 2-5A synthetase in healthy human adults before and after immunization with influenza or rubella virus vaccine.

Influenza viruses and influenza virus vaccines have been previously characterized as good inducers of IFN activity (3, 5, 13, 16, 17). This property of the virus was further demonstrated in this study by the detection of IFN and 2-5A synthetase activity in PBM cells incubated overnight with the influenza virus vaccine in vitro (Fig. 3). Furthermore, this ability to induce IFN activity was indicated in vivo by elevated levels of 2-5A synthetase activity in PBM cells of influenza virus-vaccinated humans after immunization (Fig. 1). In contrast, the rubella virus vaccine did not cause the PBM cell enzyme level to increase either in vivo 1 day postvaccination (Fig. 4) or in vitro upon overnight incubation (Fig. 5). The supernatants from these in vitro incubations contained no detectable IFN activity.

The mechanisms underlying the differential effect in IFN and 2-5A synthetase induction seen with the two virus vaccines are unclear. Contributing factors may be the relative ability of each virus to induce IFN, the physical characteristics of the vaccine (live, attenuated versus killed, subvirion), or the immunological histories of the vaccinated individuals.

Further specific changes in 2-5A synthetase levels in six of eight of the influenza virus-vaccinated individuals occurred after immunization. The enzyme activity in the PBM cells decreases relative to basal enzyme levels just before an increase on day 11 in vivo (Fig. 1). The reasons for this decrease in activity remain to be elucidated. However, the induction of 2-5A synthetase activity suggests that IFN may be produced in vivo at this time after virus immunization.

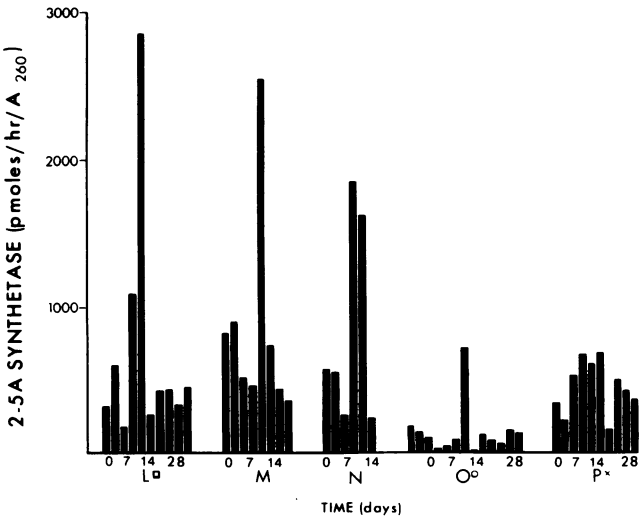

FIG. 4. In vivo 2-5A synthetase levels assayed in human PBM cells after rubella virus vaccination. PBM enzyme levels were determined at least 1 day before and twice weekly for 4 weeks after the administration of the vaccine. Day 0 indicates each individual's basal 2-5A synthetase activity level. The symbols denoting each individual are retained in Fig. 5. A_{260}, Absorbancy at 260 nm.

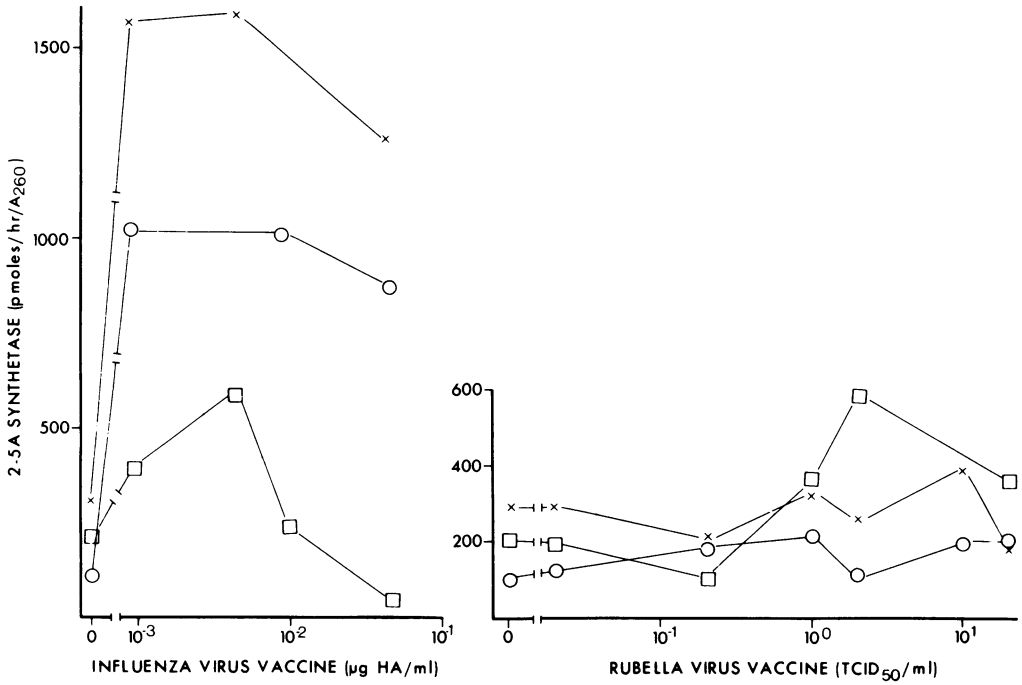

FIG. 5. Comparison of 2-5A synthetase levels assayed in PBM cells isolated 90 days postimmunization from rubella virus-vaccinated individuals after overnight incubation with the two virus vaccines. A_{260}, Absorbancy at 260 nm; HA, hemagglutinin; $TCID_{50}$, 50% tissue culture infective dose.

Knight and Fitzharris have reported that an increase in natural killer cell activity can be demonstrated with human adult PBM cells drawn 10 days postimmunization with a killed influenza virus vaccine (11). Furthermore, this increase in natural killer cell activity can be reproduced in vitro by the addition of IFN-γ to T-cell-depleted PBM cells from the vaccinated individual. Both natural killer cell activity and 2-5A synthetase activity are induced by all types (α, β, γ) of IFN in human PBM cells. It is possible, therefore, that the 2-5A synthetase activity detected in vivo approximately 11 days after virus vaccination results from IFN-γ production by human PBM cells in vivo in response to influenza and rubella virus vaccination.

In the case of rubella virus, the combination of cumulative virus replication and stimulation of the immune system (1) resulted in all five individuals showing a specific increase in 2-5A synthetase activity in vivo on day 11 after immunization (Fig. 4).

Interestingly, three members of the rubella virus-vaccinated group (L, M, N) showed physical side effects (joint pain, lymphadenopathy, rash, fever, headache) from the vaccine approximately 11 days postvaccination, and these same three individuals produced very high levels of enzyme activity on day 11 postvaccination (Fig. 4). Since similar side effects develop in humans after clinically administered IFN (22, 24), it is possible that immunization with rubella virus may induce sufficient IFN to cause the side effects in some individuals. Although the function this IFN activity may play 11 days after virus vaccination has not been elucidated, a regulatory role for IFN in antibody production has been suggested (8). From the data presented here for the rubella virus-vaccinated group, there emerges a positive correlation between the specific increase of PBM cellular 2-5A synthetase activity on day 11 postimmunization and a large increase in antibody titer. These results provide further evidence for an involvement of IFN in the human immune response to virus vaccination.

IFN itself was detectable only at low (<10 U/ml) levels in the sera of our virus-vaccinated volunteers throughout the study (data not shown). However, since the vaccination simulates only a limited viral challenge, the concentration of IFN in the serum is likely to be low and transient. Very little IFN is required to induce 2-5A synthetase production in vitro, and humans undergoing IFN therapy demonstrate increased 2-5A synthetase activity in their PBM cells which correlates with the IFN administration (19; B. Williams, unpublished data). Clearly, 2-5A synthetase is a very sensitive indicator of IFN activity. The monitoring of the enzyme in human PBM cells in this study has illustrated further the involvement of the IFN system in the response of the human body to viral antigens.

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