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2′,5′-Oligoadenylate Synthetase and Interferon in Peripheral Blood after Rubella, Measles, or Mumps Live Virus Vaccine (42586)

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Abstract. The temporal activation of the human interferon system by infection with virus was studied by serial measurements of both interferon in serum and activity of 2′,5′-oligo adenylate synthetase in peripheral mononuclear leukocytes. A frequency distribution of baseline values of synthetase was established for normal individuals. Following subcutaneous inoculation of rubella vaccine virus, serum interferon rose briefly with a peak on Day 14. The peak concentration of synthetase also occurred on Day 14 but remained elevated for greater than 1 week. After measles virus, serum interferon did not rise above baseline, but synthetase peaked on Day 14 and remained elevated. Subcutaneous inoculation of mumps vaccine virus was associated with a brief period of elevation of the synthetase and no interferon in the serum. Thus, the determination of synthetase levels in tissue may be useful in some situations to reflect a small or transient elevation of endogenous interferon.

Since its discovery by Isaacs and Lindenmann (1), the interferon system has been shown to have increasing relevance in viral diseases (2). Its importance as a natural mechanism of host defense against virus has been clearly established both in cell culture and in animals (3). In addition, the potential of exogenous interferon as a prophylactic modality in virus infection in man was first demonstrated with partially purified leukocyte interferon (4) and has been verified with interferon produced utilizing recombinant DNA technology (5). Our understanding of the mechanism of the antiviral action of interferon, although still incomplete, has been significantly enhanced by the demonstration by Kerr and Brown (6) that interferon induces cells to produce two or more enzymes, including 2′,5′-oligo adenylate (oligo(A)) synthetase, which require the presence of double-stained RNA for activation. The latter synthetase has a baseline value characteristic for each cell strain in a variety of animal species tested (7). The concentration of synthetase is greatly enhanced in lysates of cells that have received prior treatment *in vitro* or *in vivo* (in animals) with interferon (6, 8). Synthetase has also been demonstrated in the peripheral mononuclear leukocytes (PML) of normal humans and enhanced titers have been found in PML of seriously ill individuals following administration of exogenous interferon by the intravenous route (9). Raised concentrations have also been found in lysates of PML obtained during natural infection with virus and under certain other conditions (10, 11, 12).

In the present work, the frequency distribution of baseline values of 2′,5′-oligo(A) synthetase has been determined in extracts of lysates of PML of normal individuals. In addition, both the concentrations of synthetase in serial samples of PML and the titer of interferon in serial samples of sera have been determined following the administration of live attenuated rubella, measles, or mumps vaccine virus by the subcutaneous route. The results observed are described.

Methods. *Volunteers.* The normal subjects are medical student volunteers in their first year at the University of California, Irvine, California College of Medicine and healthy adult volunteers at the MRC Common Cold Unit (Salisbury, Wilts, UK).

Biologicals. Rubella live virus vaccine (Meruvax 11), mumps live virus vaccine (Mumpsvax), and measles live virus vaccine (Attenuvax) were obtained from Merck & Co., Inc. Each subject received a single vaccine virus administered by the subcutaneous route in a dose of at least one thousand tissue culture infectious doses$_{50}$ (TCID$_{50}$). In each case vaccine virus was administered to subjects whose preliminary sera lacked the specific antibody.

Interferon assay. The plaque technique

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previously described (13) utilizing human diploid fibroblasts was modified by substitution of Sindbis virus, ATC AR339, for vesicular stomatitis virus as the challenge virus. Serial fourfold dilutions of each serum were used commencing with an initial dilution of 1:10. Interferon titer was calculated in International Units utilizing NIAID reference human interferon-α Catalog No. Ga 23-901-532. The latter has an assigned titer of 4.4 $\log_{10}$ international units and an observed geometric mean titer of 5.56 ± 0.14 $\log_{10}$ laboratory units utilizing this assay.

For the preparation of extract containing 2',5'-oligo(A) synthetase, a modification of the method of Schattner was utilized (10). Mononuclear leukocytes were isolated from 2.0 ml heparinized peripheral blood by sedimentation on Ficoll–Hypaque, washed twice with phosphate buffered saline, suspended at a concentration of 10^7 cells per milliliter, pelleted, and then resuspended in an equal volume of a lytic solution (10 m*M* Hepes at pH 7.5, 90 m*M* KCl, 1.5 m*M* magnesium acetate, 0.5% (v/v) Nonidet P-40, and 10% (v/v) glycerol) and allowed to stand for 2 min. After centrifugation at 8000*g* for 6 min, the supernatant was stored at −70°C.

For the assay of 2',5'-oligo(A) synthetase activity, a modification of the method of Knight was utilized (14):

a. *Partial purification.* Extract (30 μl) was mixed with 6 μl of poly(I)poly(C) bound to finely divided cellulose (15) and incubated at room temperature for 1 hr. The poly(I)poly(C)-cellulose-bound synthetase was pelleted and washed twice at 4°C with a solution of 20 m*M* Hepes at pH 7.5, 50 m*M* KCl, 8.5 m*M* magnesium acetate, 20% glycerol (v/v), and 7 m*M* 2-mercaptoethanol.

b. *Synthesis of 2',5'-oligo(A).* The pellet was resuspended in 10 μl of a solution at 4°C of 4 m*M* adenosine triphosphate, 20 m*M* Hepes, 50 m*M* KCl, 8.5 m*M* Mg acetate, and 20% glycerol (v/v). The suspension was incubated at 30°C for 2 hr and centrifuged at 8000*g* for 6 min. The oligo(A) containing supernatant was frozen at −70°C.

c. *Measurement of 2',5'-oligo(A).* To 2.0-μl aliquots of serial dilutions of the test sample were added 5.0-μl aliquots of a postmitochondrial supernatant of an extract of Ehrlich ascites tumor cells containing high levels of the oligo(A)-binding endonuclease. After incubation at 4°C for 15 min, 13 μl of a dilution of 2',5'-adenosine tetramer 5'-triphosphate 3' ^{32}PpCp (Amersham PB 254) containing 3000 cpm were added and the mixture was spotted onto a 1-in. disk of nitrocellulose filter paper (SM 125, Sartorius, Gottingen, FRG) which was then washed twice with tap water at 25°C at the ratio of 30 ml per filter. The filters were dried and dissolved in 4 ml of Aquasol, and the activity was counted on the Beckman LS 100 scintillation counter. Controls, in quadruplicate, were run simultaneously. Controls consisted of mixtures lacking sample, but with and without endonuclease, and mixtures in duplicate, lacking sample but containing serial concentrations of a standard preparation 2',5'-oligo adenylate tetramer expressed in monphosphate equivalents. The concentration of oligo(A) in a dilution of a test sample was determined by comparing the radioactivity that had been retained on the filter with a curve generated by the radioactivity retained by the filters of the mixtures containing serial concentrations of the known standard oligo(A).

d. *Specific activity of oligo(A) synthetase.* The specific activity of synthetase was expressed as picomoles of oligo(A) generated per absorbance 260 unit per hour of incubation of the reaction.

Results. *Baseline concentrations of oligo(A) synthetase.* The geometric mean value for baseline specific activity of synthetase for the 44 normal individuals tested was 2.00 pM/A_{260}/hr with the range of one standard error from 1.45 to 2.75 pM/A_{260}/hour or an average of ±32.8%. From the frequency distribution of baseline activity in Fig. 1, it can be seen that the median value is ≤1.00 pM/A_{260}/hr but 8 of 44, or 18%, have a value of >20.0 pM/A_{260}/hr.

Interferon and oligo(A) synthetase after vaccination with attenuated vaccine viruses. From the plot of the geometric mean serum interferon for the three individuals inoculated with attenuated rubella virus in Fig. 2, it is apparent that the peak interferon titer occurred on Day 14. The serum interferon did not differ from baseline from ≤ day 12 or ≥ Day 17. The geometric mean synthetase concentration also peaked on Day 14 as can be seen in Fig. 3, but was clearly above baseline during the entire period from Days 12 to 17. The probability

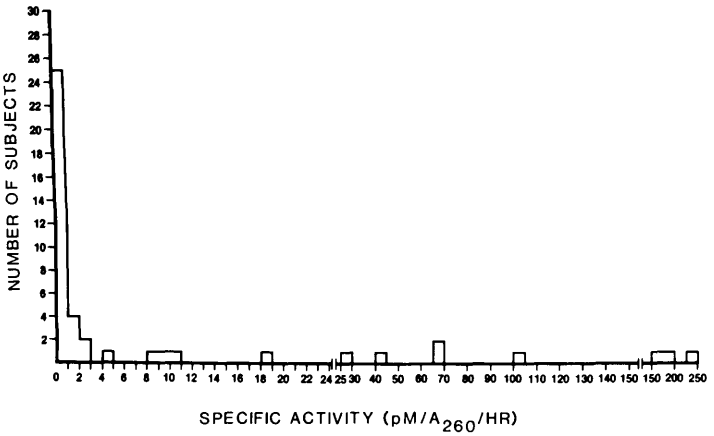

FIG. 1. Frequency distribution of oligo(A) synthetase in peripheral mononuclear cells in 44 normal human subjects.

that the synthetase activities on Days 12 and 17, respectively, differed from the baseline of Days 0 to 10 by chance was ≤ 0.025 and ≤ 0.05. After infection with either measles or mumps virus there was no detectable increase in serum interferon above baseline values. The geometric mean synthetase concentrations for three individuals infected with measles virus peaked on Day 14 (Fig. 4). For the one individual infected with mumps virus, the peak synthetase value occurred on Day 12 (Fig. 5).

Discussion. Because of the wide variety of methods for measuring 2′,5′-oligo(A) synthetase (11, 16), the variation in terminology in expressing the specific activity (7, 10), and the lot variation in activity of reagents used (9), it is not useful to compare absolute values observed in one laboratory with those from another. It would, nevertheless, be anticipated that the profile of the frequency distribution of activity in normals reported here could be duplicated by other laboratories. Although the majority of individuals have a low value of synthetase, at least 18% have a concentration of well over 10 times either the geometric mean or median values. It is possible that normal individuals with elevated concentrations of synthetase actually have subclinical infections but such has not yet been proven. Thus, at the present time, there is some hazard in utilizing an isolated value of synthetase activity to decide that an acute virus infection is or is not present.

In the present work subcutaneous inoculation of rubella virus has been associated with a rise in oligo(A) synthetase that peaked at 14

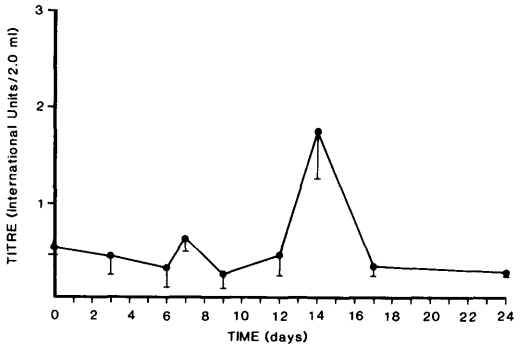

FIG. 2. Serum interferon following infection with rubella live virus vaccine. Geometric means and standard error of titers for three individuals following subcutaneous inoculation of a dose of at least 1000 $TCID_{50}$.

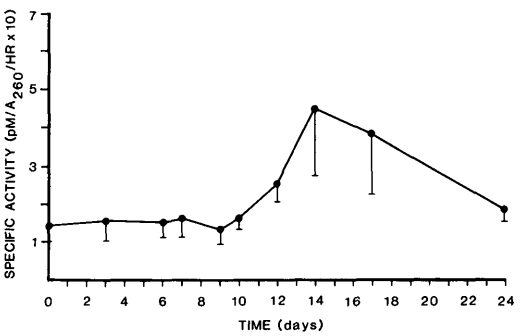

FIG. 3. Oligo(A) synthetase in peripheral mononuclear cells following inoculation with rubella live virus vaccine. Geometric mean and standard error of activities for three individuals under conditions noted in Fig. 2.

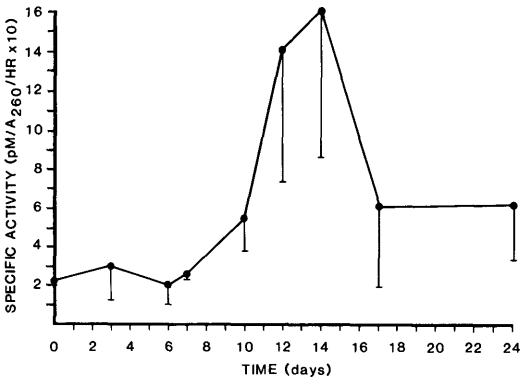

FIG. 4. Oligo(A) synthetase in peripheral mononuclear cells following inoculation with measles live virus vaccine. Geometric mean and standard error of activities for three individuals following subcutaneous inoculation of a dose of at least 1000 $TCID_{50}$.

days and lasted about 2 weeks. In the only other report of serial determinations of oligo(A) synthetase in PML during viral infection in man, Penn and Williams demonstrated a peak concentration 11 days after subcutaneous inoculation with rubella live virus vaccine (12). The difference in peak concentration from the present report is not clear but may be due to the presence of interfering enteroviruses (the current vaccinations occurred in the summer) or a difference in the inoculum of viable virus. Schattner *et al.* (17) have shown a peak of PML synthetase on Days 1 and 4, respectively, after intraperitoneal inoculation of either 10^7 plaque-forming units (PFU) vesicular stomatitis virus or 2×10^4 PFU Sindbis virus. In the present study vaccination with rubella virus was also associated with a rise in detectable serum interferon. It should be noted that the duration of elevation of detectable interferon was much briefer than the period of elevation of oligo(A) synthetase in PML. This discrepancy probably reflects the short half-life of interferon, *in vivo,* and may be due, in part, to an inactivator that has been described in serum and other body fluids, *in vitro* (18). In the current report, subcutaneous inoculation of attenuated measles virus was also associated with an increase in PML synthetase activity but not with a detectable rise in serum interferon. It would appear that the Attenuvax vaccine strain of measles virus induces less interferon than does the original Edmonstron strain, since the latter was associated with high levels of serum interferon (19). Nevertheless, the induction of interferon by the current strain of attenuated measles virus is presumably reflected by the prolonged elevation of oligo(A) synthetase in PML. Subcutaneous inoculation of a single individual with attenuated mumps virus was associated with a much briefer elevation of oligo(A) synthetase and again with no elevation of serum interferon. Thus for all three viruses the titer of synthetase in PML remained elevated for a longer period than the titer of interferon in serum. This discrepancy has at least two reasonable explanations. In the first explanation, which might be called the "central" theory, one must assume a relatively rapid turnover between peripheral and local mononuclear leukocytes. One can hypothesize that the synthetase detected in PML is induced while the leukocytes are central in lymph nodes or other tissues where virus infection induces local interferon production to a much greater degree than is reflected by the level of interferon in the peripheral blood serum. In the second explanation, which might be called the "peripheral" theory, one can hypothesize that the synthetase detected in PML is induced peripherally while the leukocytes are exposed to interferon in the peripheral blood. Since interferon in the serum is continuously inactivated, while the induced enzyme in PML is

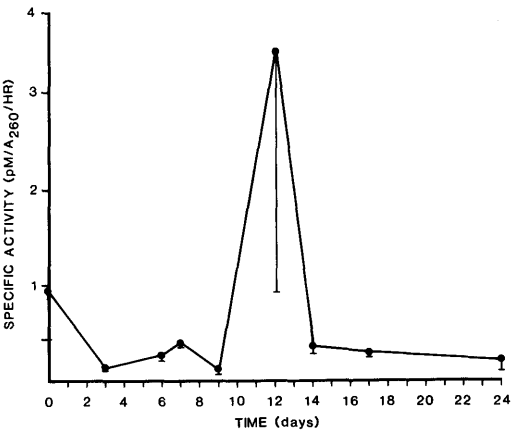

FIG. 5. Oligo(A) synthetase in peripheral mononuclear cells of a single individual inoculated with mumps live virus vaccine. Geometric mean and standard error of two determinations for each sample.

not, a low-level increase in serum interferon might be masked while one of its actions, progressively increasing synthetase, becomes apparent. Whether either or both of these theories are correct remains to be proven. In either case, it appears that, under appropriate circumstances, serial determinations of oligo(A) synthetase in PML will be generally useful in reflecting small or transient elevations of endogenous interferon.

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