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Lymphocytic 2′,5′-Oligoadenylate Synthetase Activity Increases prior to the Appearance of Neutralizing Antibodies and Immunoglobulin M and Immunoglobulin G Antibodies after Primary and Secondary Immunization with Yellow Fever Vaccine

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Primary and secondary immunizations with live, attenuated yellow fever virus vaccine (17D strain) were performed in order to study the course of appearance of virus-neutralizing antibodies and immunoglobulin M (IgM) and IgG antibodies directed against the virus and the interferon-dependent enzyme 2′,5′-oligoadenylate synthetase (2′,5′AS) activity, determined in homogenates of peripheral B and T lymphocytes. From cellular ATP, this enzyme generates 2′,5′-oligoadenylates which mediate degradation of viral mRNA by stimulation of a latent RNase. By day 4 after the first immunization, the earliest and highest 2′,5′AS activity was present in the T-lymphocyte fraction. By day 7, the enzyme activity was highest in the B-lymphocyte fraction. Virus-neutralizing antibodies appeared on day 7, and IgM antibodies were present on day 12. After the second immunization, performed 2 years ± 2 months later, the only significant increase in 2′,5′AS activity was observed in the T-lymphocyte fraction. Virus-neutralizing antibodies were present from day 1, whereas no IgM antibodies were detected. By day 12, 80% of the vaccinees were IgG positive. In the primary and secondary (memory) immune responses, 2′,5′AS activity is expressed in the T-lymphocyte fraction prior to the appearance of antibodies directed against the virus and may serve as an early and sensitive marker of an ongoing virus infection which is otherwise difficult to detect. No changes in conventional laboratory analysis parameters, such as in differential blood cell counts or total IgA, IgG, and IgM, disclosed the immune activity in either the primary or the secondary immunization.

The immune defense against viruses is dependent on production of several lymphokines, such as interferons (IFNs) (2, 34, 39, 42) and interleukins (10, 18, 25, 35–37) released from antigen-presenting cells and T-helper lymphocytes. In the primary recognition and elimination of viruses, the activation of IFN-responsive genes is an early event (9, 12, 23, 32). Within a few hours after infection, the mRNA activity related to these genes is increased (9, 19, 22). One of the transcribed and translated gene products is the IFN-stimulated and double-stranded-RNA-dependent enzyme 2′,5′-oligoadenylate synthetase (2′,5′AS) (7, 8, 32). From cellular ATP, this enzyme generates 2′,5′-oligoadenylates, which are diester condensation products with different chain lengths (oligomers) (21). The produced oligomers (dimers and trimers, etc.) activate an endogenous latent RNase with mRNA- and virus-degrading activities. The end results of these processes are destruction of viral mRNA and shutdown of cellular protein synthesis (41).

The time course of appearance of various immune components in viral immune defense adheres to the following scheme. First, there are a virus replication state and an antigen recognition phase, both closely connected to a release of IFN (28). Within a couple of days, increased natural killer cell activity is detected (17, 25). In addition, after a lag period of about 4 days, cytotoxic T cells are activated and immunoglobulin M (IgM) antibodies appear (27). A different course is taken by immune reactions to reinfections or revaccinations, characterized by memory response (15, 16, 20, 26, 30). Within 2 to 5 days, memory T cells, situated in regional lymph nodes, recognize antigens that have been presented to them previously, and memory B lymphocytes rapidly reinstitute the production of IgG molecules (16, 27, 37).

In light of the rapidly growing recognition of the family of lymphokines (1, 34, 37), the involvement of these response modifiers in primary and secondary immune reactions requires further examination. However, this is difficult because of the brief periods of in vivo turnover (a few minutes) characteristic of IFNs, making it necessary to use other indicators of IFN-induced effects. It has been shown that the IFN-induced enzyme 2′,5′AS is a useful marker for monitoring IFN-related activities (3, 4, 31, 33, 38). Typically, the enzyme is highly activated in viral infections during which alpha IFN (IFN-α) is synthesized and released by peripheral blood lymphocytes (PBL) (11, 39, 42). We took advantage of the much longer decay time for enzymatic activity than for lymphokines, allowing an extension of the interval during which immune activity can be measured.

As an immunogen, we used live, attenuated yellow fever virus (YFV), which enabled us to study the immune response in individuals who were exposed to this virus for the first time. After primary immunization, 2′,5′AS activity was detected in T

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and B cells before the appearance of virus-neutralizing antibodies and before IgM could be detected. T lymphocytes appear to be the cells principally responsible for the early 2′,5′AS activity increase. Secondary immunization, 2 years later, again resulted in an increase in 2′,5′AS activity, this time, however, significantly detectable only in T lymphocytes and unaffected by the presence of neutralizing antibodies to YFV.

MATERIALS AND METHODS

Research design and participants. (i) Primary immunization. Healthy individuals, four females (22 to 40 years old) and four males (23 to 35 years old), were recruited from the staff at the hospital laboratory of Odense University Hospital and vaccinated with YFV (Arilvax, 17D strain; Wellcome, Beckenham, United Kingdom).

(ii) Secondary immunization. Healthy individuals, eight females (24 to 51 years old) and six males (27 to 45 years old), were recruited and vaccinated as described above and revaccinated with YFV vaccine 2 years ± 2 months after receiving the primary vaccination. Six individuals had received the primary immunization as participants in another study by the Department of Clinical Chemistry, Odense University Hospital. These studies were approved by the regional ethical committee (Funen and Vejle counties, Denmark) and followed the Helsinki II declaration.

Chemicals and reagents. Poly(I-C) and ATP were from Pharmacia (Uppsala, Sweden). Acid alumina WA-1, bovine serum albumin (BSA), and alkaline phosphatase were obtained from Sigma (St. Louis, Mo.), and columns for ion-exchange chromatography were obtained from Bio-Rad (Richmond, Calif.). Creatine kinase and creatine phosphate were obtained from Boehringer GmbH (Mannheim, Germany), Nonidet P-40 was obtained from Calbiochem-Behring (La Jolla, Calif.), and [α-^{32}P]ATP was obtained from Amersham (Buckinghamshire, England). IFN-α was generously supplied by Hoffmann-La Roche (Basel, Switzerland). Determination of protein in cell homogenates was done with bicinchoninic acid reagent (Pierce, Rockford, Ill.).

Blood sampling. Blood samples were obtained on day 1 before primary or secondary immunizations and on days 4, 7, 11, 12, and 14 after vaccination. The following hematological parameters were measured at each sampling: sedimentation rate and total leukocytes; granulocytes, lymphocytes, eosinophils, monocytes, and basophils (counted by the same laboratory technician throughout the study); and serum IgG, IgM, and IgA, determined by using a Cobas Fara centrifugal analyzer (Roche, Basel, Switzerland).

Analysis of IgM and IgG antibodies to YFV. Antibodies against YFV were measured by an antibody capture enzyme-linked immunosorbent assay technique, as described in detail elsewhere (29). Tests for IgM and IgG antibodies were considered positive when the test serum/control serum optical density ratios were ≥2.0; control sera were obtained from people without a history of YFV immunization or other known exposure to flaviviruses.

Detection of antibodies to YFV by neutralization tests. As described elsewhere (24), the serum dilution-plaque reduction neutralization (PRN) test measures the ability of a serum to reduce the amount of infectious virus in a given dose. For our tests, we used Vero cells and about 100 PFU of YFV. The titer was considered the reciprocal of the highest dilution of serum inhibiting ≥90% of plaques.

Preparation of PBL and B and T lymphocytes. Two milliliters of heparinized venous blood was layered on top of 3 ml of Ficoll-Hypaque (Nycomed, Copenhagen, Denmark) and centrifuged (800 × *g*) at room temperature for 20 min. The interphase was transferred to conical tubes and washed three times with 2 ml of RPMI 1640 medium (Gibco, Roskilde, Denmark) containing 1% BSA, pH 7.4. A fourth wash was done with RPMI 1640 medium without BSA. Cell lysis was achieved by incubation at 4°C in 0.5% (wt/vol) Nonidet P-40 for 45 min. Homogenates were stored at −80°C until tested.

B and T lymphocytes were prepared by the immune rosetting technique, as described previously (14). Final separation was done by Ficoll-Hypaque gradient centrifugation. B cells were collected from the top of the gradient. T cells were collected by centrifugation following lysis of sheep erythrocytes. Homogenates were prepared as described above. Before analysis of 2′,5′AS activity, all homogenates were adjusted to a final protein concentration of 1 mg/ml because higher protein concentrations inhibit enzyme activity (2a). Separation of lymphocytes into subtypes was controlled by FACScan analysis (Becton Dickinson Immunocytometry Systems, San Jose, Calif.); T and B cells were labelled with monoclonal antibodies against CD2 and CD19 cell surface markers, respectively (Becton Dickinson). For both lymphocyte subtypes, the purity obtained was verified to be >95%. The viability of the cells was always >98% (trypan blue exclusion test).

Analysis of 2′,5′AS. 2′,5′AS was analyzed as previously described (5). In brief, 10 μl of PBL homogenates (corresponding to about 10^5 cells) was incubated at 37°C for 105 min in 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 8) containing 2.5 mM ATP, 20 mM magnesium acetate, 10 mM creatine phosphate, 3 mg of creatine kinase per ml, 10% glycerol, 200 μg of poly(I-C) (synthetic double-stranded RNA) per ml, and 2.66 kBq of [α-^{32}P]ATP per tube. Radioactive phosphorus not incorporated into the diester bonds of the synthesized 2′,5′-oligoadenylates was removed with alkaline phosphatase. Oligoadenylates were separated from free radioactive ATP by using 400-μl acid alumina columns thoroughly washed with a 1 M glycine-HCl buffer (pH 2). The 2′,5′AS activity was expressed in units per milligram of protein or as percent change, with units defined as nanomoles of ATP converted per minute. A 3-ml volume of the glycine buffer was eluted directly into plastic vials, and radioactivity in the ^{3}H channel was determined by Cerenkov radiation in a Packard (Tricarb) scintillation counter.

Incubation of PBL with IFN-α. Isolated PBL or B or T lymphocytes (about 10^6 cells per ml) were incubated in a 95% O_2–5% CO_2 atmosphere at 37°C for 18 h in RPMI 1640 medium with 10% fetal calf serum. IFN-α was added in increasing amounts (0 to 500 U/ml). Incubations were terminated by repeated washings in RPMI 1640 medium, and cellular lysates were prepared as described above.

Statistical methods. Total biological variation was defined as analytical day-to-day variation plus interindividual variances. Levels of significance were evaluated by Student's *t* test.

RESULTS

YFV vaccination. None of the subjects had previously been vaccinated with YFV. Three individuals had slight fevers 5 or 6 days after receiving the first dose of vaccine, but none of the other vaccinees had adverse reactions to either vaccine dose.

Blood samples. Primary YFV vaccination induced only slight biological day-to-day variations in total leukocyte counts, sedimentation rate, and IgA, IgM, or IgG levels. In eight individuals, differential counts of leukocytes showed no biologically significant changes in percentages of granulocytes (52 to 57%), lymphocytes (34 to 40%), monocytes (5 to 7%), eosinophils (2 to 5%), or basophils (0.8 to 1.0%). Percentages express the range of values for all the individuals in the experimental period of 2 weeks. Similarly, slight variations were observed in the 14 individuals revaccinated after 2 years. Differential counts showed the following variations: granulocytes, 59 to 61%; lymphocytes, 30 to 33%; monocytes, 4 to 6%; eosinophils, 2 to 3%; and basophils, 0.8 to 0.9%.

Neutralizing antibodies after primary vaccination with YFV. The presence of IgM, IgG, and neutralizing antibodies against YFV was determined for specimens collected before vaccination on day 1 and with specimens collected on days 4, 7, and 12. IgM antibodies against YFV were present on day 12 in five of six serum specimens (Table 1), but this was not reflected in the PRN test. IgG antibodies were found in one subject on day 1; another subject became borderline positive 12 days after vaccination. PRN tests were positive for seven of eight subjects by day 7. By day 12, seven of eight subjects had seroconverted.

Neutralizing antibodies after revaccination with YFV. IgM antibodies against YFV were not detected in serum samples from revaccinated subjects (Table 1); in contrast, 2 of 10 samples had IgG antibodies. These two serum specimens came from the two subjects who were IgG positive in the primary vaccination. Positivity increased until 8 of 10 had IgG antibodies by day 12. The PRN test was positive for 9 of 10 subjects on day 1; by day 7, all sera were positive (Table 1).

2′,5′AS activity in PBL and in B and T lymphocytes. The 2′,5′AS activity on the indicated days relative to day 1 for homogenates of PBL and B and T lymphocytes is shown in Fig. 1. For comparative and statistical reasons, each value was expressed as a ratio, i.e., the experimental-day value divided by the day 1 value, meaning that each individual serves as its own control. On day 4, the ratio of 2′,5′AS activity (mean ± standard deviation) in PBL homogenates increased to 2.80 ± 0.65 (from 0.98 ± 0.78 to 2.76 ± 2.02 U/mg; total biological variation, 80% [not significant]). For B lymphocytes, the 2′,5′AS activity ratio increased to 1.80 ± 0.47 (from 1.35 ± 0.87 to 2.40 ± 1.62 U/mg; total biological variation, 66% [not significant]). However, for T lymphocytes, the 2′,5′AS activity ratio increased significantly to 2.60 ± 1.50 (from 0.80 ± 0.42 to 2.06 ± 1.20 U/mg; total biological variation, 58%; $P < 0.05$). On day 7, the 2′,5′AS activity was further increased in all homoge-

TABLE 1. IgM, IgG, and neutralizing (PRN) antibodies after vaccination with YFV

Immunization and antibody type	No. of subjects with antibody/total on day[a]:			
	1[b]	4	7	12
Primary				
IgM	0/6	0/6	0/6	5/6
IgG	1/8	1/8	1/8	2/8
PRN	0/8 (<10)	0/8 (<10)	2/8 (<10–40)	7/8 (<10–160)
Secondary				
IgM	0/6	0/6	0/6	0/6
IgG	2/10	2/10	3/10	8/10
PRN	9/10 (10–1,280)	9/10 (10–1,280)	10/10 (10–1,280)	10/10 (20–1,280)

[a] Number of subjects with antibodies against YFV or neutralizing (PRN) antibodies in relation to total number of tested subjects. Positivity for IgG and IgM antibodies was defined at a test serum/control serum optical density ratio of ≥2.0. Values in parentheses are PRN titers. The acceptance limit for positive titers in this assay was 10. See Materials and Methods for details on the PRN test and the number of participants. Serum samples for antibody determination were not available for all participants.

[b] Values on day 1 were obtained before vaccination.

nates. Enzyme activity peaked on day 7 and declined to near-baseline values by day 14.

The 2′,5′AS activity was only moderately changed by revaccination with YFV (Fig. 1). It was clear, however, that by day 4 after vaccination, T lymphocytes had a statistically significant net increase in 2′,5′AS activity to 1.55 ± 0.74 (from 0.84 ± 0.44 to 1.31 ± 0.69 U/mg; total biological variation, 53%; $P < 0.05$) and a higher specific activity level than B lymphocytes. Notably, on day 1, 36% of the total 2′,5′AS activity came from the T lymphocytes and 64% came from the B lymphocytes. On day 4, however, T lymphocytes increased their fractional 2′,5′AS activity to 53%, whereas the activity of B lymphocytes dropped to 47%. For the remainder of the observation period, only insignificant fluctuations in the activities of the enzyme were seen (Fig. 1).

Effect of IFN-α on PBL. Before primary YFV immunization, isolated PBL that had been incubated overnight in vitro with 0 to 500 U of IFN-α per ml showed a dose-dependent increase in 2′,5′AS activity (Fig. 2a); the maximum increase was 266%. In contrast, isolated PBL stimulated with IFN-α on days 7 and 14 had a markedly reduced response, with a maximum increase of 56 to 79% (not statistically significant; total biological variation, 90%). Before the secondary immunization, the maximum increase at 500 U of IFN-α per ml was 272%. However, on days 7 and 14, isolated PBL were unaffected by addition of IFN-α (Fig. 2b), with maximum increases in 2′,5′AS activity of 238% on day 7 and 215% on day 14.

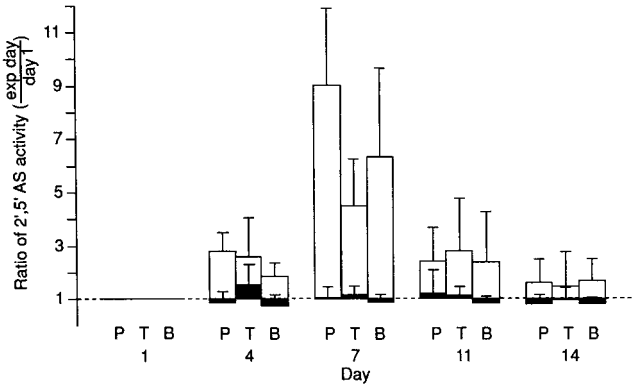

FIG. 1. Mean ratios of 2′,5′AS activity (experimental day/day 1 activity ratio) in PBL (P), T lymphocytes (T), and B lymphocytes (B) after primary (open bars) and secondary (filled bars) YFV vaccinations. Means ± 1 standard deviation are shown.

DISCUSSION

In order to achieve an immunogenic state, several lymphokines are released in response to contact with antigen (1, 6, 8, 37). Among these, the IFNs induce IFN-responsive genes after binding to specific cell surface receptors (9, 12, 13, 22, 23, 32), and several proteins are synthesized. Thus, transcription of the 2′,5′AS gene leads to production of an enzyme directed at degradation of virus particles. We have here demonstrated that 2′,5′AS activity increases by day 4 after primary vaccination and reaches a maximum on day 7, coinciding with the appear-

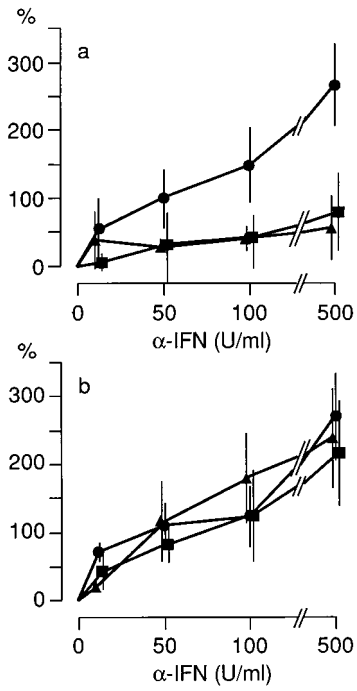

FIG. 2. Ficoll-Hypaque-isolated PBL from primary (a) ($n = 8$) and secondary (b) ($n = 16$) YFV-vaccinated subjects were incubated with IFN-α overnight. 2′,5′AS activity in PBL homogenates collected on days 1 (●), 7 (▲), and 14 (■) was determined. Means ± 1 standard deviation are shown.

ance of neutralizing antibodies and, if present, with mild clinical signs. We chose YFV as an immunogen in order to exclude the possible influences of memory lymphocytes or preexisting YFV-neutralizing antibodies. Furthermore, measurement of 2′,5′AS activity was selected for the following reasons. First, 2′,5′AS activity is the major biologically active factor directed at virus degradation. Second, it is possible to monitor variations in enzyme activities within a much broader time frame than can be done with lymphokines because of their high turnover. The latter point makes it very difficult to collect meaningful data in situations in which the time courses of lymphokine production are not known. Third, the 2′,5′AS activity data can be helpful in defining the optimal sampling time for lymphokine release in infectious diseases. The 2′,5′AS enzyme activity in homogenates from different cell types is easily measured (3, 4, 7, 33). In order to measure the free enzyme in serum, specific antibodies are needed. We are in the process of producing such antibodies.

In other studies, peak 2′,5′AS activity occurred much later (11 to 14 days) after inoculation of mumps, rubella, measles, or influenza virus (31, 38). Obviously, differences in inoculum size and type of virus are of significance in both the character and the magnitude of the response. Nevertheless, it is difficult to interpret results of immunization when the preimmune status of the vaccinees is not known (38). The importance of these factors is underlined by our finding of highly different responses in primary and secondary immunizations. YFV-neutralizing antibodies were detected from day 7 in the primary vaccination; an increase in IgM antibodies followed. One individual was positive for IgG antibodies from day 1. Most likely, this was due to a previous exposure to virus from the flavivirus family, but we had no record on this point. The PRN titers appeared to peak on day 12. Only two individuals showed a further increase prior to the second immunization. IgM antibodies were not detected after revaccination, but the IgG antibodies were significantly increased from day 7, and neutralizing antibodies were detectable throughout. Thus, after 2 years, neutralizing antibodies to YFV are present in sera of vaccinees, and a rapid memory response from IgG-producing B lymphocytes occurs. The lymphocyte subfraction(s) responsible for memory response is unknown (16, 26, 30, 37), nor is it known whether perpetuated antigen presentation is necessary for continuous production of antibodies. It has been suggested that T-helper cells or follicular dendritic cells in regional lymph nodes present information to the B lymphocytes locally (15), with plasma cells then differentiating or proliferating from them (27, 30, 37).

Detection of small, immunologically active subfractions of T lymphocytes in peripheral blood may be inherently difficult because of the lack of cell-specific surface antibodies or simply because there are too few cells present. Our findings point to the existence of a subfraction of activated T cells which can be detected early in an immunization course by determining 2′,5′AS activity in PBL. It appears that within the first 4 days after primary vaccination and also revaccination with YFV, circulating peripheral T lymphocytes are the most active with respect to 2′,5′AS activity. Therefore, a detailed description of cell surface markers of the lymphocyte subtypes that show increased 2′,5′AS activity might provide information about the phenotype of T cells responsible for memory response. Characterization of T cells into subsets with distinct cytokine secretory patterns (37) will be helpful in understanding how and in which cell types the IFN-2′,5′AS system is activated in the viral immune defense. Also, our current investigations show that stimulation of PBL with YFV in vitro induces increases in 2′,5′AS activity (unpublished data). This finding can be utilized in further characterization of which cell types are directly sensitive to the exposure to virus.

Recently, it was demonstrated that there exists a direct correlation between cell surface IFN-α receptors and enzymatic activity of 2′,5′AS (13). It was further shown that specific IFN-α receptor down-regulation resulted when PBL were exposed to IFN-α in vitro (13). After primary vaccination in this study, the 2′,5′AS response was markedly reduced on days 7 and 14 when PBL were incubated in vitro with increasing IFN-α concentrations. This suggests that YFV vaccination stimulated production of sufficient amounts of IFN-α to cause down-regulation of IFN receptors. In contrast, after in vitro stimulation 4, 7, and 14 days after the second dose of YFV, PBL showed full responsiveness to IFN-α in 2′,5′AS activity, indicating that free IFN had not down-regulated IFN receptors to any significant degree after revaccination.

Questions therefore arise as to whether and to what extent cytokines are involved in responses to reexposure to virus antigens when preexisting virus-neutralizing antibodies are present and as to how antigen recognition is affected by such reexposure. It has been demonstrated that lymphocytes collected from individuals immunized with measles virus have a cytotoxic potential unrestricted by and independent of IgG antibodies directed against the virus (6). This cytotoxicity is related to the release of cytokines involved in the antigen presenting process (3, 40) by secretion from macrophages or from dendritic cells (9). Our study seems to support these findings, because T cells were activated in terms of 2′,5′AS activity in spite of the presence of virus-neutralizing antibodies.

We conclude that lymphocytic 2′,5′AS enzyme in immunocompetent cells is activated early in both primary and secondary immunizations against YFV and precedes the appearance of virus-neutralizing and IgM and IgG antibodies. Lymphocytic 2′,5′AS enzyme activity therefore acts as a sensitive indicator of ongoing virus infection. We suggest that after revaccination, 2′,5′AS activity might be induced by other cytokines, because there were no indications of IFN-α release in terms of effect on IFN receptors in that situation. Potentially, the 2′,5′AS system could be used in diagnosis and monitoring of primary or secondary viral infections.

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REFERENCES

1. **Akira, S., T. Taga, and T. Kishimoto.** 1993. Interleukin-6 in biology and medicine. Adv. Immunol. **54:**1–78.
2. **Balkwill, F. R.** 1989. Interferons. Lancet **i:**1060–1063.
2a. **Bonnevie-Nielsen, V.** Submitted for publication.
3. **Bonnevie-Nielsen, V., A.-M. Gerdes, J. Fleckner, J. S. Petersen, B. Michelsen, and T. Dyrberg.** 1991. Interferon stimulates the expression of 2′,5′-oligoadenylate synthetase and MHC class I antigens in insulin-producing cells. J. Interferon Res. **11:**255–260.
4. **Bonnevie-Nielsen, V., I. Heron, T. Kristensen, B. Michelsen, and Å. Lernmark.** 1989. Postimmunization activity of oligoadenylate synthetase in peripheral blood lymphocytes from healthy individuals. J. Clin. Lab. Immunol. **28:**155–160.
5. **Bonnevie-Nielsen, V., M. L. Larsen, J. J. Frifelt, B. Michelsen, and Å. Lernmark.** 1989. Association of IDDM and attenuated response of 2′,5′-oligoadenylate synthetase to yellow fever vaccine. Diabetes **38:**1636–1642.
6. **Casali, P., and M. B. A. Oldstone.** 1982. Mechanisms of killing of measles virus-infected cells by human lymphocytes: interferon associated and unassociated cell-mediated cytotoxicity. Cell. Immunol. **70:**330–344.
7. **Chebath, J., P. Benech, A. Hovanessian, J. Galabru, and M. Revel.** 1987. Four different forms of interferon-induced 2′,5′-oligo(A) synthetase identi-

fied by immunoblotting in human cells. J. Biol. Chem. **262:**3852–3857.
8. **Cohen, B., Y. Gothelf, D. Vaiman, L. Chen, M. Revel, and J. Chebath.** 1991. Interleukin-6 induces the (2′,5′)-oligoadenylate synthetase gene in M1 cells through an effect on the interferon-responsive enhancer. Cytokine **3:**83–91.
9. **Cohen, B. D., D. Peretz, P. Benech, and J. Chebath.** 1988. Enhancer-like interferon responsive sequences of the human and murine (2′,5′) oligoadenylate synthetase gene promoters. EMBO J. **7:**1411.
10. **Dinarello, C. A.** 1989. Interleukin-1 and its biologically related cytokines. Adv. Immunol. **44:**153–205.
11. **Friedman, R. M.** 1977. Antiviral activity of interferons. Bacteriol. Rev. **41:** 543–567.
12. **Gariglio, M., S. Panico, G. Gribaudo, M. G. Martinotti, G. Cavallo, and S. Landolfo.** 1991. Activation of interferon-inducible genes in vivo by synthetic double-stranded RNA, poly rI:rC. Microbiologica **14:**179–183.
13. **Gerdes, A.-M., M. Hørder, and V. Bonnevie-Nielsen.** 1992. Gene dosage and down-regulation of the α-interferon receptor. Scand. J. Clin. Lab. Invest. **52:**189–192.
14. **Gmelig-Meyling, F., and R. E. Ballieux.** 1977. Simplified procedure for the separation of human T and non-T cells. Vox Sang. **33:**5–8.
15. **Gray, D., M. Kosco, and B. Stockinger.** 1991. Novel pathways of antigen presentation for the maintenance of memory. Int. Immunol. **3:**141–148.
16. **Gray, D., and P. Matzinger.** 1991. T cell memory is short-lived in the absence of antigen. J. Exp. Med. **174:**969–974.
17. **Herberman, R. B., and J. R. Ortaldo.** 1981. Natural killer cells: their role in defenses against disease. Science **214:**24–30.
18. **Hilbert, D. M., M. P. Cancro, P. A. Scherle, R. P. Nordan, J. van Snick, W. Gerhard, and S. Rudikoff.** 1989. T cell derived IL-6 is differentially required for antigen-specific antibody secretion by primary and secondary B cells. J. Immunol. **143:**4019–4024.
19. **Hovanessian, A. G.** 1991. Interferon-induced and double-stranded RNA-activated enzymes: a specific protein kinase and 2′,5′-oligoadenylate synthetases. J. Interferon Res. **11:**199–205.
20. **Jamieson, B. D., and R. Ahmed.** 1989. T cell memory. Long-term persistence of virus-specific cytotoxic T cells. J. Exp. Med. **169:**1993–2005.
21. **Justesen, J., D. Ferbus, and M. N. Thang.** 1980. Elongation mechanism and substrate specificity of 2′,5′-oligoadenylate synthetase. Proc. Natl. Acad. Sci. USA **77:**4618–4622.
22. **Kerr, I. M., and G. R. Stark.** 1991. The control of interferon-inducible gene expression. FEBS Lett. **285:**194–198.
23. **Levy, D. E., D. J. Lew, T. Decker, D. S. Kessler, and J. E. Darnell, Jr.** 1990. Synergistic interaction between interferon-α and interferon-γ through induced synthesis of one subunit of the transcription factor ISGF3. EMBO J. **9:**1105–1111.
24. **Lindsey, H. S., C. H. Calisher, and J. H. Mathews.** 1976. Serum dilution neutralization test for California group virus identification and serology. J. Clin. Microbiol. **4:**503–510.
25. **Luger, T. A., J. Krutmann, R. Kirnbauer, A. Urbanski, T. Schwarz, G. Klappacher, A. Köck, M. Micksche, J. Malejczyk, E. Schauer, L. T. Man, and P. B. Sehgal.** 1989. IFN-β_2/IL-6 augments the activity of human natural killer cells. J. Immunol. **143:**1206–1209.
26. **Mackay, C. R., W. L. Marston, and L. Dudler.** 1990. Naive and memory T cells show distinct pathways of lymphocyte recirculation. J. Exp. Med. **171:** 801–817.
27. **MacLennan, I. C. M., and D. Gray.** 1986. Antigen-driven selection of virgin and memory B cells. Immunol. Rev. **91:**61–85.
28. **Metz, D. H.** 1975. The mechanism of action of interferon. Cell **6:**429–439.
29. **Monath, T. P., R. R. Nystrom, R. E. Bailey, C. H. Calisher, and D. J. Muth.** 1984. Immunoglobulin M antibody capture enzyme-linked immunosorbent assay for diagnosis of St. Louis encephalitis. J. Clin. Microbiol. **20:**784–790.
30. **Oehen, S., H. Waldner, T. M. Kündig, H. Hengartner, and R. M. Zinkernagel.** 1992. Antivirally protective cytotoxic T cell memory to lymphocytic choriomeningitis virus is governed by persisting antigen. J. Exp. Med. **176:** 1273–1281.
31. **Penn, L. J. Z., and B. G. R. Williams.** 1984. Interferon-induced 2-5A synthetase activity in human peripheral blood mononuclear cells after immunization with influenza virus and rubella virus vaccines. J. Virol. **49:**748–753.
32. **Revel, M., and J. Chebath.** 1986. Interferon-activated genes. Trends Biochem. Sci. **11:**166–170.
33. **Schattner, A., D. Wallach, G. Merlin, T. Hahn, S. Levin, B. Ramot, and M. Revel.** 1982. Variation of (2′,5′)-oligo A synthetase level in lymphocytes and granulocytes of patients with viral infections and leukemia. J. Interferon Res. **2:**355–361.
34. **Sen, G. C., and P. Lengyel.** 1992. The interferon system. J. Biol. Chem. **267:**5017–5020.
35. **Sims, J. E., M. A. Gayle, J. L. Slack, M. R. Alderson, T. A. Bird, J. G. Giri, F. Colotta, F. Re, A. Mantovani, K. Shanebeck, K. H. Grabstein, and K. Dower.** 1993. Interleukin 1 signaling occurs exclusively via the type I receptor. Proc. Natl. Acad. Sci. USA **90:**6155–6159.
36. **Street, N. E., and T. R. Mosmann.** 1991. Functional diversity of T lymphocytes due to secretion of different cytokine patterns. FASEB J. **5:**171–177.
37. **Swain, S. L., L. M. Bradley, M. Croft, S. Tonkonogy, G. Atkins, A. D. Weinberg, D. D. Duncan, S. M. Hedrick, R. W. Dotton, and G. Huston.** 1991. Helper T-cell subsets: phenotype, function and the role of lymphokines in regulating their development. Immunol. Rev. **123:**115–144.
38. **Tilles, J. G., F. Balkwill, and J. Davilla.** 1987. 2′,5′-Oligoadenylate synthetase and interferon in peripheral blood after rubella, measles, or mumps live virus vaccine. Proc. Soc. Exp. Biol. Med. **186:**70–74.
39. **Torrence, P. F.** 1982. Molecular foundations of interferon action. Mol. Aspects Med. **5:**129–171.
40. **Wallach, D., M. Fellous, and M. Revel.** 1982. Preferential effect of γ interferon on the synthesis of HLA antigens and their mRNAs in human cells. Nature (London) **299:**833–836.
41. **Williams, B. R. G., I. M. Kerr, C. S. Gilbert, C. N. White, and L. A. Ball.** 1978. Synthesis and breakdown of pppA2′p5′A2′p5′A and transient inhibition of protein synthesis in extracts from interferon-treated and control cells. Eur. J. Biochem. **92:**455–462.
42. **Yang, K., H. Samanta, J. Dougherty, B. Jayaaram, R. Broeze, and P. Lengyel.** 1981. Interferons, double-stranded RNA, and RNA degradation. J. Biol. Chem. **256:**9324–9328.