Continuous Production of Interferon in Normal Mice: Effect of Anti-interferon Globulin, Sex, Age, Strain and Environment on the Levels of 2-5A Synthetase and p67K Kinase

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SUMMARY

Two interferon-mediated enzymes, a 2-5A synthetase and a kinase that phosphorylates a 67000 mol. wt. (p67K) protein were found at variable levels in different organs of mice. Among the different strains of mice included in this study, germ-free mice had the lowest levels of these enzymes. The levels of 2-5A synthetase and p67K kinase were enhanced significantly in all mice following treatment with mouse (α + β) interferon. Here, we show that the presence of 2-5A synthetase and p67K kinase in different organs of normal mouse (untreated) was due, at least in part, to a constant production of interferon under different physiological conditions. Accordingly, injection of normal mice with anti-mouse interferon (α + β) globulin led to a significant decrease in the level of 2-5A synthetase and p67K kinase. In conventional mice (C3H/He), the level of both of these enzymes was higher in female than in male animals and was decreased with age or when such animals were reared isolated in a pathogen-free protected unit. The levels of 2-5A synthetase and p67K kinase were also decreased in normal mice following injection with a powerful antibiotic against a very wide spectrum of Gram-positive and Gram-negative bacteria. These results suggest that the production of interferon was induced continuously in normal mice. Such induction was mediated by both internal and external agents.

INTRODUCTION

Treatment of cells with interferon results in the induction of two dsRNA-dependent enzymes, pppA(2'p5'A), synthetase (2-5A synthetase) and a specific protein kinase (for references, see Hovanessian, 1979). The protein kinase activity is manifested by the phosphorylation of an endogenous 67000 mol. wt. protein (p67K kinase) in mouse cells or a 72000 mol. wt. protein (p72K kinase) in human cells. Both the 2-5A synthetase and the protein kinase are present in different organs of mice and their level is enhanced several-fold after treatment of mice with interferon or after injection of inducers of interferon, such as viral and synthetic dsRNAs (Krishnan & Baglioni, 1980; Hovanessian & Rivièrè, 1980; Hovanessian et al., 1981b; Krust et al., 1982). Accordingly, enhanced levels of these enzymes in different organs of mice have been used as convenient markers for the presence and action of interferon in an organism (Saron et al., 1982; Rivièrè & Hovanessian, 1983).

Interferon is not detectable routinely in the sera of normal mice which show considerable levels of 2-5A synthetase and p67K kinase. This is not surprising in view of the fact that circulating interferon has a very short half-life and, furthermore, the level of interferon produced under normal physiological and environmental conditions is too low to be detectable in an antiviral assay in vitro (Bocci, 1981). In contrast, enhanced levels of 2-5A synthetase and p67K kinase last several hours and it is possible to assay efficiently any modification in the levels of these enzymes (Hovanessian et al., 1981b, 1983). In addition to these, during repeated
treatments with interferon, the levels of 2-5A synthetase and p67K kinase remain constantly enhanced (Youn et al., 1983). Here, we provide evidence to suggest that interferon is produced in normal mice and that such interferon is responsible, at least in part, for the presence of these dsRNA-dependent enzymes in different organs of mice.

**METHODS**

*Materials.* \[^{[\gamma-32P]}ATP and \[^{3H}]ATP were supplied by Amersham. Poly(I)-poly(C)-Sepharose was prepared as described previously (Hovanessian et al., 1983). Poly(A)-poly(U) was obtained from P-L Biochemicals. The antibacterial agent cefotaxime (Claforan) was obtained from Roussel (France). Cefotaxime is a \(\alpha\) isoform of a new methoxy-imino cephalosporin. This bacteriocidal agent is active against a very wide spectrum of Gram-positive and Gram-negative bacteria at a dose to animals of 50 mg/kg/day (Chabbert & Lutz, 1981).*

*Preparation and titration of interferon.* Mouse \((\alpha + \beta)\) interferon was prepared by infection of mouse C-243 cells with Newcastle disease virus and was purified on a column of CM-Sepharose (Krust et al., 1982). The partially purified preparation of interferon had a specific activity of \(5 \times 10^7\) units/mg protein. The interferon activity was assayed by inhibition of vesicular stomatitis virus cytopathic effect in mouse L-929 cells. One NIH unit of mouse interferon was equivalent to 10 effective laboratory units.

*Mice.* Pathogen-free C3H/He, BALB/c, DBA/2 and C57BL/6 mice were obtained from Institut Pasteur, Paris. AKR, Swiss nu/+ (mice heterozygous for the nude gene) and Swiss nu/nu mice (nude mice homozygous for the nude gene) were from Iffa Credo, L'Arbresle, France. Nude mice were maintained in a pathogen-free environment. Germ-free mice (C3H/He Pas.) were the generous gift of J. L. Guénet (Institut Pasteur, Paris) and were reared in a laminar air-flow hood during the experiment.

*Serum and preparation of tissue extracts.* After ether anaesthesia, mice were bled from the axillary vessels and the blood was collected in polystyrene tubes and left 60 min at room temperature. Serum was then collected after centrifugation (500 \(g\), 15 min) and stored at \(-80^\circ\mathrm{C}\). Frozen tissues were homogenized mechanically (Polytron, type PT 10/35, Kinematica, Switzerland) in low-salt buffer (10 mM-HEPES pH 7.6, 10 mM-KCl, 2 mM-magnesium acetate, 7 mM-2-mercaptoethanol and aprotinin at 100 units/ml). This suspension was left for 15 min at 4 \(^\circ\mathrm{C}\) before addition of NP40 at a final concentration of 0.5%. After 15 min, each suspension was sonicated for 10 s and centrifuged at 5000 \(g\) for 20 min. Tissue extracts were stored at \(-80^\circ\mathrm{C}\).

*Assay of 2-5A synthetase.* Assay of 2-5A synthetase was in a total mixture (600 \(\mu\)l) containing 200 \(\mu\)l of tissue extract (1 mg of tissue), 20 mM-HEPES pH 7.6, 50 mM-KCl, 25 mM-magnesium acetate, 7 mM-2-mercaptoethanol, 5 mM-ATP, 10 mM-creatine phosphate, 0.16 mg/ml creatine kinase, 0.1 mg/ml poly(I)-poly(C) and 5 \(\mu\)l of \[^{3H}]ATP (0.2 \(\mu\)Ci/ml) which was purified in this laboratory (Buffet-Janvresse et al., 1983). Incubation was for 90 min at 30 \(^\circ\mathrm{C}\) and was terminated by heating at 90 \(^\circ\mathrm{C}\) for 5 min. \(^3\)H-labelled 2-5A was purified by DEAE-cellulose chromatography as described previously (Buffet-Janvresse et al., 1983). The concentration of 2-5A in AMP c.p.m. The 2-5A synthetase levels were calculated on this basis and are given as units corresponding to 1 nmol of 2-5A synthesized per mg of tissue per h.

*Assay of protein kinase.* The p67K kinase activity in tissue extracts was assayed after partial purification on poly(I)-poly(C)-Sepharose in the presence of l-arginine (50 to 100 units/ml) as described by Hovanessian et al. (1983). Phosphorylation (90 min, 30 \(^\circ\mathrm{C}\)) was with 1 \(\mu\)M-(\(\gamma-32\)P)ATP (25 Ci/mmol) in HEPES buffer/glycerol (10 mM-HEPES pH 7.6, 50 mM-KCl, 5 mM-magnesium acetate, 10 mM-MnCl\(_2\), 7 mM-2-mercaptoethanol, 20% glycerol). Samples were heated in electrophoresis sample buffer and analysed on polyacrylamide slab gels (10%) containing SDS as described previously (Hovanessian et al., 1983). All the samples were supplemented with bovine plasma albumin (10 \(\mu\)g) before electrophoresis. The \(^{32}\)P-labelled 67000 molecular weight proteins were localized in the stained gels, according to the mobility of albumin (69000 mol. wt.) and pieces of gel (0.5 cm) containing the \(^3\)P-labelled proteins were cut and the radioactivity measured by liquid scintillation. The results are given as fmol PO\(_4\) incorporated into the 67000 mol. wt. protein per mg of tissue per h.

*Preparation of anti-mouse \((\alpha + \beta)\) interferon globulin in rabbits.* Partially purified mouse \((\alpha + \beta)\) interferon (5 \(\times 10^7\) NIH units/mg protein) was used to immunize a female rabbit (3.5 kg, Fauve de Bourgogne obtained from Vannier, Mont-Moreau, France). Before each injection, partially purified mouse interferon was mixed with 10 mg of commercially available rabbit albumin (Sigma). The immunization was by eight weekly injections of the antigen: four injections subcutaneously, each with 1.5 \(\times 10^9\) NIH units of interferon; two injections intravenously each with 1.5 \(\times 10^9\) NIH units of interferon supplemented with 5 mg poly(A)-poly(U); two injections intravenously of 2.5 \(\times 10^9\) NIH units of interferon and 5 mg poly(A)-poly(U). Rabbits were bled regularly from the marginal ear vein for titration of antibody production. Anti-mouse \((\alpha + \beta)\) globulin production became detectable after the fifth injection of antigen. Eight days after the last immunization, the rabbit was sacrificed and the serum was collected and heat-treated at 56 \(^\circ\mathrm{C}\) for 30 min. The neutralizing titre of the anti-interferon globulin was \(5 \times 10^{-4}\) against 5 NIH units of mouse \((\alpha + \beta)\) interferon.
Production of interferon in normal mice

Table 1. Circulating interferon and the levels of 2-5A synthetase and p67K kinase in normal C3H/He mice

<table>
<thead>
<tr>
<th>IFN (NIH units/ml)</th>
<th>2-5A synthetase (nmol AMP/mg/h)</th>
<th>Protein kinase (fmol PO₄/mg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3 (146)†</td>
<td>47.2 ± 10.5</td>
<td>215 ± 37</td>
</tr>
<tr>
<td>20-40 (8)</td>
<td>76.8 ± 23.4</td>
<td>388 ± 51</td>
</tr>
<tr>
<td>41-60 (6)</td>
<td>112.2 ± 42.5</td>
<td>548 ± 76</td>
</tr>
</tbody>
</table>

* Mice were grown in the same environment. The levels of 2-5A synthetase (in the spleen) and p67K kinase (in the lung) are given as the mean ± S.D.
† Number of mice is given in parentheses.

Preparation of anti-mouse (α + β) interferon globulin in sheep. Mouse (α + β) interferon (2.5 × 10⁸ to 5.0 × 10⁸ NIH units/mg protein) was used to immunize a sheep (male, 60 kg, breed Ile de France, selected by Dr Salmon, I.N.R.A., Grignon, France). Before each injection, mouse interferon was mixed with 20 mg sheep albumin (prepared in this laboratory). The immunization was by eight weekly intravenous injections of the antigen: the first injection was 12 × 10⁶ units of interferon supplemented with 20 mg poly(A)-poly(U); the second and third injections were 12 × 10⁶ units of interferon; the fourth injection was 36 × 10⁶ units of interferon supplemented with 20 mg poly(A)-poly(U) and was administered intravenously, as well as 40 mg poly(A)-poly(U) (4 mg/ml) injected subcutaneously; the fifth, sixth and seventh injections were with 12 × 10⁶ units of interferon supplemented with 12 mg poly(A)-poly(U); the eighth injection was with 36 × 10⁶ units of interferon supplemented with 20 mg poly(A)-poly(U). Anti-mouse (α + β) globulin production was detectable after the fourth injection. Sera collected 1, 2 and 3 weeks after the last immunization were pooled and heat-treated at 56 °C for 30 min. The immunoglobulin fractions were then separated by ammonium sulphate (40%) precipitation and dialysed extensively against phosphate-buffered saline. The neutralizing titre of the anti-interferon globulin was 1 × 10⁻⁵ against 5 NIH units of mouse (α + β) interferon.

Statistical analysis. The difference between experimental and control groups was analysed by Student’s t-test.

RESULTS

Level of circulating interferon in comparison with the level of 2-5A synthetase and p67K kinase in normal mice

2-5A synthetase and p67K kinase are present in different organs of normal C3H/He mice (Krust et al., 1982). The levels of these enzymes vary from one organ to another and there is no consistent correlation between them. Among the different organs studied, the lung has the highest level of p67K kinase activity whereas the spleen shows the highest level of 2-5A synthetase. For this reason, therefore, both of these organs were investigated in this study.

In most normal mice, the level of circulating interferon is too low (<3 NIH units/ml) to be detectable in an antiviral assay in vitro (see Methods). In contrast, however, such mice show considerable levels of 2-5A synthetase and p67K kinase (Table 1). In a group of 160 C3H/He mice (all normal females, 2 months old) which were studied in several experiments over a 1 year period, we found some mice with detectable levels of circulating interferon (20 to 60 NIH units/ml). Such mice represented less than 10% (14 out of 160 mice) of the population studied. The levels of 2-5A synthetase and p67K kinase were higher in the two groups of mice with circulating interferon compared with those which did not show detectable levels of interferon (Table 1). This circulating interferon was neutralized by anti-mouse (α + β) interferon globulin.

Levels of 2-5A synthetase and p67K kinase are decreased in normal mice injected with anti-interferon globulin

In order to illustrate that the presence of 2-5A synthetase and p67K kinase in mice is a consequence of interferon production under conventional breeding conditions, we injected normal mice with rabbit serum containing anti-mouse (α + β) interferon immunoglobulin (Methods). The control group received serum from an unimmunized rabbit. Table 2 indicates that the levels of both 2-5A synthetase and p67K kinase were decreased significantly in normal mice that received anti-mouse interferon globulin compared with corresponding controls. The level of 2-5A synthetase was also investigated in mice injected with a preparation of sheep anti-
Fig. 1. Decrease in the level of splenic 2-5A synthetase in mice injected with anti-mouse (α + β) interferon globulin. Six female mice (C3H/He), 2 months old, were investigated in each group. Mice were injected intravenously, twice at 48 h intervals with 2, 5, 10, 50 or 100 µl of sheep anti-mouse (α + β) interferon globulin fraction (abscissa) diluted in phosphate-buffered saline (200 µl). Control mice were injected with 200 µl phosphate-buffered saline. The neutralizing titre of the anti-interferon globulin was $1 \times 10^{-5}$ against 5 units of mouse (α + β) interferon. Five days after the first injection, mice were sacrificed and the level of splenic 2-5A synthetase was assayed. The values are given as the mean ± S.D. (ordinate). The differences between enzyme levels of mice injected with anti-interferon globulin and control mice were significant ($P < 0.02$, $P < 0.02$, $P < 0.01$, $P < 0.01$ and $P < 0.001$ for 2, 5, 10, 50 and 100 µl of anti-interferon globulins, respectively). The protein concentration of the anti-mouse interferon globulin fraction was 50 mg/ml.

Fig. 2. Variation in the level of 2-5A synthetase in the spleen of normal mice with ageing. Female mice (C3H/He) at different ages were injected intravenously with $10^5$ NIH units of mouse (α + β) interferon (as in Table 3). Twenty-four h later, five control (not treated with interferon, □) and five interferon-treated (○) mice were sacrificed and the levels of 2-5A synthetase in the spleen were assayed. The values represent the means of five independent assays.

Table 2. Inhibitory effect of anti-interferon globulin on the levels of 2-5A synthetase and protein kinase in normal mice*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2-5A synthetase (nmol AMP/mg/h)</th>
<th>Protein kinase (fmol PO₄/mg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>59.2 ± 16.8</td>
<td>268 ± 43</td>
</tr>
<tr>
<td>Control serum</td>
<td>5 µl/mouse</td>
<td>54.3 ± 12.6</td>
</tr>
<tr>
<td></td>
<td>20 µl/mouse</td>
<td>56.7 ± 11.9</td>
</tr>
<tr>
<td>Anti-IFN serum</td>
<td>5 µl/mouse</td>
<td>24.9 ± 7.7 ($P &lt; 0.001$)</td>
</tr>
<tr>
<td></td>
<td>20 µl/mouse</td>
<td>14.1 ± 5.5 ($P &lt; 0.001$)</td>
</tr>
</tbody>
</table>

* Six female mice (C3H/He), 2 months old, were investigated in each group. Mice were injected intravenously, daily for 5 days with 5 or 20 µl of rabbit serum diluted in phosphate-buffered saline (100 µl). Control serum was from an unimmunized normal rabbit. Anti-IFN serum was from a rabbit producing anti-mouse (α + β) interferon globulin. The neutralizing titre of the anti-interferon globulin was $5 \times 10^{-4}$ against 5 units of mouse (α + β) interferon. Mice were sacrificed on the 6th day and the level of 2-5A synthetase (in the spleen) and p67K kinase (in the lung) was assayed. The values are given as the mean ± S.D. $P$: the difference between enzyme levels of mice injected with anti-IFN serum and control serum was analysed by Student's $t$-test.

mouse (α + β) interferon globulin (Methods). This sheep antibody was prepared by immunization with a highly purified fraction of mouse (α + β) interferon ($2.5 \times 10^8$ to $5.0 \times 10^8$ NIH units/mg protein). Fig. 1 shows that the level of 2-5A synthetase was decreased significantly in mice injected with the sheep anti-mouse interferon globulin. These results (Table 2 and Fig. 1) indicate that the presence of 2-5A synthetase and p67K kinase in mice is due to the
production of (α + β) interferon under normal conditions. Variations in the level of these enzymes in normal mice might be used, therefore, in order to obtain a fair estimate of the concentration of interferon.

Levels of 2-5A synthetase and p67K kinase in different strains of mice

The levels of these interferon-mediated enzymes are variable in different strains of normal mice: C3H/He, BALB/c, C57BL/6, DBA/2, AKR, Swiss nu/+ , Swiss nu/nu and C3H/He germ-free (Table 3). Among these different strains, germ-free mice have the lowest levels. Both enzymes are enhanced significantly 24 h after injection of mice with (α + β) interferon. It is worthwhile to note that mice with high levels of 2-5A synthetase are not necessarily the ones which have high levels of p67K kinase. This difference emphasizes the fact that the inductions of these interferon-mediated enzymes are not always correlated. This has been reported in cell cultures in vitro (Wood & Hovanessian, 1979; Hovanessian et al., 1981a).

In view of the very low levels of 2-5A synthetase in the spleen of normal germ-free mice, we investigated two other tissues which in conventional mice are under constant stimulation for the production of locally produced interferon: the lung and the intestines. It is considered that in these 'external' anatomical surface areas, mucosae are infiltrated with lymphoid cells which are good producers of interferon in response to continuous contact with bacteria, viruses, toxins and other agents (Bocci, 1981). Table 4 shows that the level of 2-5A synthetase is high in the intestines and in the lungs of normal conventional mice. In contrast, enzyme levels in both of these organs of germ-free mice are too low to be detectable by our assay.

Effect of environment on the level of 2-5A synthetase and protein kinase in normal C3H/He mice

The fact that the levels of interferon-mediated enzymes were very low in germ-free mice suggested that this might be due to the reduced production of interferon because of the germ-free conditions (environment and food) in which the animals were reared. The effect of environment
Table 5. Levels of interferon-mediated enzymes in normal mice reared under different conditions*

<table>
<thead>
<tr>
<th>Environment</th>
<th>Food</th>
<th>2-5A synthetase (nmol AMP/mg/h)</th>
<th>Protein kinase (fmol PO_4~/mg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal laboratory</td>
<td>Conventional</td>
<td>62.5 ± 15.4</td>
<td>361 ± 54</td>
</tr>
<tr>
<td>Biochemistry laboratory</td>
<td>Conventional</td>
<td>41.7 ± 11.3</td>
<td>211 ± 22</td>
</tr>
<tr>
<td>Protected unit</td>
<td>Sterile</td>
<td>28.9 ± 8.2</td>
<td>157 ± 16</td>
</tr>
</tbody>
</table>

*(p < 0.001) (P < 0.001)

* Six female mice (C3H/He), 2 months old, were studied in each group. Mice were kept for 2 weeks in the animal laboratory, in a biochemistry laboratory or in a pathogen-free protected unit (Iffa Credo) with air ventilation and filtration systems. Mice in the protected unit were fed with autoclaved water and irradiated food (pathogen-free). Two weeks later, mice were sacrificed and the levels of 2-5A synthetase (in the spleen) and p67K kinase (in the lung) were assayed. The results are presented as the mean ± s.D. P: the difference between enzyme levels of mice kept at the biochemistry laboratory or in the protected unit and animal laboratory was analysed by Student’s t-test.

Table 6. Levels of 2-5A synthetase and p67K kinase in the spleens of normal C3H/He mice injected with cefotaxime*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2-5A synthetase (nmol AMP/mg/h)</th>
<th>Protein kinase (fmol PO_4~/mg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>32.5 ± 7.9</td>
<td>188 ± 41</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>6.8 ± 3.2 (P &lt; 0.001)</td>
<td>65 ± 20 (P &lt; 0.001)</td>
</tr>
</tbody>
</table>

* Six male mice (C3H/He), 2 months old, were investigated in each group. Mice were injected intramuscularly with cefotaxime (1 mg/mouse) administered at 12 h intervals. Three days later, mice were sacrificed and the levels of 2-5A synthetase (in the spleen) and p67K kinase (in the lung) were assayed. The values are given as the mean ± S.D.

was investigated in conventional C3H/He mice reared under different conditions: in an animal laboratory where normal uninfected mice and rats were kept, in a biochemistry laboratory where several persons carried out their daily research work and in a protected unit with air ventilation and filtration systems. After 2 weeks, the levels of 2-5A synthetase and p67K kinase were assayed (Table 5). Mice reared in the protected unit with sterile feeding showed a significant decrease in the level of their enzymes compared to the controls which were in the animal laboratory. Interestingly, mice kept in the biochemistry laboratory also had significantly decreased levels of these enzymes compared with the controls, although both groups of mice were fed similarly. These results emphasize the role of the environment in providing agents responsible for the induction of interferon.

Effect of bacteriocidal agent cefotaxime on the level of 2-5A synthetase and protein kinase in normal C3H/He mice

Germ-free mice lack the intestinal microbial flora which probably plays a role in the induction of interferon in conventional mice (Hudson & Luckey, 1964; Bocci, 1981). For this reason, normal C3H/He mice were injected with cefotaxime (Chabbert & Lutz, 1981). This product is active against a very wide spectrum of Gram-positive and Gram-negative bacteria and at a dose of 1 mg/mouse/24 h will destroy the microbial flora. Table 6 gives the levels of 2-5A synthetase and p67K kinase in normal mice injected with cefotaxime. Three days after the injection, the level of these interferon-mediated enzymes was reduced significantly (P < 0.001).

Variations in the levels of 2-5A synthetase between male and female mice and with ageing

The levels of 2-5A synthetase in the spleens of female mice were significantly higher than those of male mice of the same age group (Table 7). In both male (data not shown) and female (Fig. 2) mice, the levels of these enzymes were low in the newly born mice but were enhanced significantly in mice 3 weeks old. These enhanced levels lasted for 4 to 5 weeks and then decreased in older mice. Whatever the level of 2-5A synthetase in any age group, however, treatment with interferon enhanced it by comparable levels.
Table 7. Levels of 2-5A synthetase in the spleen of female and male mice of different age groups*

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Female (nmol AMP/mg/h)</th>
<th>Male (nmol AMP/mg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-23</td>
<td>58.1 ± 23.6 (P &lt; 0.001)</td>
<td>45.5 ± 7.3</td>
</tr>
<tr>
<td>27</td>
<td>74.9 ± 23.3 (P &lt; 0.001)</td>
<td>37.4 ± 8.7</td>
</tr>
<tr>
<td>31-32</td>
<td>74.9 ± 21.3 (P &lt; 0.001)</td>
<td>30.1 ± 8.9</td>
</tr>
<tr>
<td>35</td>
<td>39.5 ± 12.8 (P &lt; 0.01)</td>
<td>29.7 ± 10.1</td>
</tr>
<tr>
<td>39-40</td>
<td>34.5 ± 15.8 (P &lt; 0.01)</td>
<td>26.9 ± 10.9</td>
</tr>
<tr>
<td>45-48</td>
<td>70.3 ± 31.2 (P &lt; 0.001)</td>
<td>35.8 ± 8.3</td>
</tr>
</tbody>
</table>

* Six mice were studied in each age group. The levels of 2-5A synthetase were assayed in extracts of spleen as described. P: the difference between the level of 2-5A synthetase in female and male mice in each age group was analysed by Student’s t-test.

DISCUSSION

The production of interferon in normal mice has been suggested previously but no direct evidence has been provided (Bocci, 1981). Here, we show that interferon is produced in normal mice and this interferon is responsible, at least in part, for the presence of enhanced levels of 2-5A synthetase and p67K kinase in different organs of mice. By the use of anti-mouse (α + β) interferon globulin, we show that most of this endogenous interferon is of the α + β type since the levels of 2-5A synthetase and p67K kinase were decreased significantly in normal mice which received anti-interferon globulin. It is also interesting to note that when circulating interferon was detectable in some normal mice (Table 1), all of this interferon activity was neutralized in vitro by an anti-(α + β) interferon serum. Our results, however, do not rule out completely the possibility that γ interferon might also be produced under normal conditions.

The level of 2-5A synthetase and p67K kinase in different organs of normal mice could be used conveniently to assess the production of interferon under different physiological influences such as sex, environment and ageing. The early increase in the levels of interferon-mediated enzymes in the young mice might be correlated with the establishment of intestinal microbial flora in these mice at 2 to 3 weeks of age, a period which might also coincide with a decrease in the level of maternally transmitted immunoglobulins (Hudson & Luckey, 1964; Drasar & Hill, 1974; Clover & Zarkower, 1980; Inoue et al., 1980). On the other hand, the decrease in the level of enzymes in the older mice is not clear. Since the response to exogenous interferon is almost identical in older and younger mice, then the lower level of interferon-mediated enzymes in older mice might reflect lower production of interferon under normal conventional breeding conditions. Higher levels of 2-5A synthetase and p67K kinase were observed in female mice compared to male mice. This is probably due to higher levels of interferon production in female mice. Accordingly, Zawatzky et al. (1982) have recently shown that X-linked loci, in addition to other autosomal loci, are involved in regulation of interferon production in the mouse. These authors have also shown that higher levels of interferon are produced in female than in male mice in response to inoculation by different viruses (Zawatzky et al., 1983).

Among the different strains of mice included in this study, germ-free mice (C3H/He) showed the lowest levels of 2-5A synthetase and p67K kinase. This reflects low levels of interferon produced in these mice due to the absence of normal microbial flora and as well as to the absence of external inducing agents, since germ-free mice are reared under sterile conditions. The respective roles of the microbial flora and the environment in providing agents responsible for the production of interferon was investigated by experiments shown in Tables 5 and 6. The continuous production of interferon in conventional C3H/He mice was decreased when these...
mice were injected with a powerful bacteriocidal agent or when they were reared isolated in a protected unit under pathogen-free conditions.

These results provide biochemical evidence to show that the production of interferon is induced continuously in normal mice. The role of this interferon produced in normal mice is not yet clear, but it is tempting to speculate that it might be functional in different processes such as in growth and in differentiation. It might also be responsible for mediating an antiviral activity expressed in health (for review, see Bocci, 1981). In accordance with this latter, Belardelli et al. (1984) have shown that injection of mice with anti-mouse (\(\alpha + \beta\)) interferon renders peritoneal macrophages permissive to infection by vesicular stomatitis virus or encephalomyocarditis virus. The peritoneal macrophages in normal mice therefore develop an antiviral state due to the action of endogenous interferon.

We are grateful to Dr V. Bocci for critical reading of this manuscript.

REFERENCES


