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Induction of indoleamine 2,3-dioxygenase in mouse lung during virus infection

(inflammation/serotonin/superoxide/influenza)

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ABSTRACT **Indoleamine 2,3-dioxygenase [indoleamine: oxygen 2,3-oxidoreductase (decyclizing)] activity in the supernatant fraction (30,000 × *g*, 30 min) of mouse lung homogenate increased approximately 120-fold after infection with PR8 influenza virus. Both specific and total enzyme activities started to increase linearly from the 5th day after infection, reached the highest level around the 11th day, and then gradually decreased to normal values in about 3 weeks. Other enzymes in the lung, such as certain lysosomal enzymes and monoamine oxidase, did not change significantly throughout the experiments. The time course of the increase in the enzyme activity was quite different from that of virus replication in the lung (a peak by the 3rd day and persistence until the 9th day) or that of serum antibody content (started to rise on the 9th day). Rather, it appeared to be closely related to the infiltrations of mononuclear and lymphocytic cells. When mice were exposed to a higher dose of virus and did not recuperate, the time course of the increase of the enzyme activity was essentially identical to that seen with a low concentration of virus. A maximum stimulation of the enzyme activity in the lung occurred on the 9th day after infection; the increase was approximately 100-fold. However, serum antibody content was slight and virus titer in the lung remained high.**

Indoleamine 2,3-dioxygenase [IDO; indoleamine:oxygen 2,3-oxidoreductase (decyclizing)] is a heme-containing enzyme (1) that catalyzes the incorporation of the superoxide anion as well as molecular oxygen (2, 3) into the pyrrole moiety of various indoleamine derivatives, such as tryptophan and serotonin (4, 5). In contrast to tryptophan 2,3-dioxygenase, which catalyzes a similar reaction but is present exclusively in the liver (6), IDO is ubiquitously distributed in various organs of mammals, including brain, lung, spleen, alimentary canal, and epididymis (7), but its biological significance is not yet clearly understood. Recent studies from our laboratory demonstrated that the enzyme is dramatically induced in the mouse lung after a single intraperitoneal injection of bacterial endotoxin (8). Endotoxin, the lipopolysaccharide fraction (LPS) obtained from the cell wall of Gram-negative bacteria, is an inflammatory agent and induces nonspecific immune responses (9). The effect appeared to be specific for the pulmonary IDO activity because in all other tissues tested no significant increase in the enzyme activity was observed. In order to determine whether or not the pulmonary IDO activity is altered by specific infection of the lung, mice were exposed to PR8 influenza virus. In this paper, we report that the pulmonary IDO activity was increased more than 100-fold around the 11th day after virus infection.

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MATERIALS AND METHODS

Materials. DL-[*ring*-2-^{14}C]Tryptophan [35 Ci/mol (1 Ci = 3.7 × 10^{10} becquerels)] was purchased from Schwarz/Mann and the D and L isomers were separated by cellulose column chromatography with a solvent system of *n*-butyl alcohol/pyridine/water (4:4:1, vol/vol). Catalase was a product of Boehringer Mannheim and was dialyzed to remove a thymol preservative. Methylene blue, ascorbic acid, and L-tryptophan were obtained from Wako (Osaka, Japan). Paraquat (methyl viologen) was a generous gift of L. L. Smith (Central Toxicology Laboratory, Alderley Park, Nr. Macclesfield, Cheshire, England). All other chemicals were of reagent grade.

Animals. All animals used in this study were male slc:ICR mice weighing 33 ± 2 g, and were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). All mice were then raised in an isolator in an air-conditioned room at 25°C and 50% relative humidity, in the laboratory of the Institute for Virus Research, Kyoto University.

Virus Infection. Influenza/AO/PR8/34 (HON1) virus was kindly donated by T. Iwasaki of the Institute for Virus Research, Kyoto University. Mice were exposed in a glass chamber to virus aerosol with a nebulizer, containing 10^6 50% egg infectious doses (10^6 EID_{50}) or $10^{7.5}$ EID_{50} per 0.1 ml of 3% calf serum/Eagle's minimum essential medium for 30 min at a pressure of 0.5 atmosphere (50 kPa). Control mice were exposed to aerosol without the virus.

Various Assays. Blood samples were taken by the cardiac puncture under ethyl ether anesthesia at various intervals after infection for assay of serum antibody. Lungs were removed, weighed, and stored frozen at −70°C for subsequent assays of virus and the IDO activity or immersed in a formalin solution for histological examination. Other tissues were also rapidly removed and frozen on dry ice and stored at −70°C for subsequent assay of the IDO activity. Except for epididymis, the tissues were homogenized with 2 vol of ice-cold 0.14 M KCl/0.02 M potassium phosphate, pH 7.0, by using a Kinematica Polytron homogenizer (Lucerne, Switzerland). The epididymis was homogenized with 10 vol of the same buffer. Homogenates were centrifuged at 30,000 × *g* for 30 min. The resulting supernatants were used as the enzyme source. The IDO activity was assayed as described (7). Other lung suspensions were prepared and assayed for virus content in chicken eggs. Virus was detected in these cultures by the hemadsorption technique (10). Titers were calculated by the method of Reed and Muench (11) and expressed as EID_{50} per 0.1 ml of lung suspensions. The

Abbreviations: IDO, indoleamine 2,3-dioxygenase; LPS, lipopolysaccharide; EID_{50}, dose infectious for 50% of eggs tested.

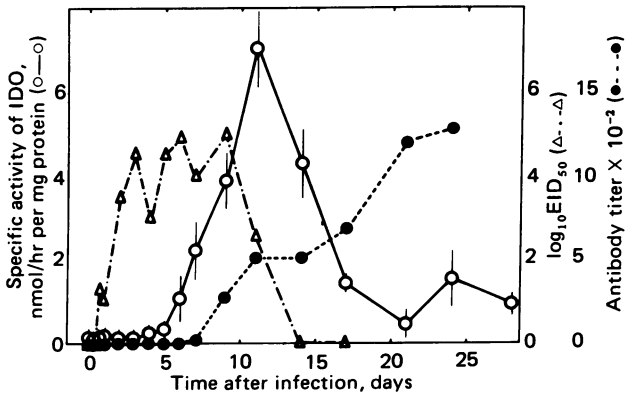

FIG. 1. Virus titer and IDO activity in the lung and serum antibody content at intervals after infection with 10^6 EID_{50} per 0.1 ml of PR8 virus. ○, Pulmonary IDO activity; △, virus infectivity titer in the lung; ●, antibody titer in serum. Enzyme activity represents the mean ± SEM for six mice.

antibody titers of sera were estimated by means of the hemagglutination inhibition method in Takatsy microplates (10). β-Glucuronidase, acid phosphatase, and monoamine oxidase activities were determined by the method of Levry and Marsh (12), Schmidt (13), and Guilbault *et al.* (14), respectively. Protein concentration was determined by the method of Lowry *et al.* (15) with bovine serum albumin as a standard.

RESULTS

When mice were exposed in a glass chamber to influenza virus aerosol (10^6 EID_{50} per 0.1 ml), virus replication began within 24 hr after infection, reached a peak by the 3rd day, and persisted until the 9th day (Fig. 1). Rapid elimination of the virus from the lung commenced thereafter and on the 14th day practically none was found. The specific activity of IDO in the lung increased almost linearly from the 5th day after infection. The enzyme activity was usually at the highest level (100- to 120-fold) around the 11th day and then gradually decreased to normal values in about 3 weeks. Fig. 1 also shows that the detectable serum antibody did not appear until the 9th day after infection, when the virus had started to disappear. The level of serum antibody rose thereafter, and was detectable in dilutions up to 1:1024 on the 24th day.

The time course of the changes in the IDO activity in various tissues of mice was determined after virus infection (10^6 EID_{50}/0.1 ml). As shown in Table 1, the effect of virus infection appeared to be specific for the lung and trachea. The specific activity of pulmonary IDO increased approximately 120-fold around the 11th day after virus infection. In trachea, a similar change in the IDO activity was observed, although the plateau was maintained between the 9th and the 11th day. In other tissues, the IDO activity did not change significantly, except in the pancreas the IDO activity was significantly increased (about 40-fold) on the 9th day after infection. Hepatic tryptophan 2,3-dioxygenase did not change significantly. Other enzymes in the lung such as β-glucuronidase, acid phosphatase (typical lysosomal enzymes), and monoamine oxidase (another enzyme involved in the metabolism of biogenic amines) did not change significantly (data not included). These results were essentially identical with those observed with LPS as an inducer (8), suggesting that the increase in this enzyme activity was probably due to net synthesis of protein.

Macroscopically about 10–20% of the lung was consolidated on the 5th day, then the percentage of these lesions gradually increased, and on the 9th day about 70% of the lung had lesions. After the 17th day regeneration was evident and on the 24th day there was no longer apparent macroscopic change in the lung.

In histological studies, changes in the lungs of these PR8 influenza virus-infected mice were almost the same as those observed with influenza viruses previously (17). In the present study, there was no obvious histological change in the lung until the 4th day. After the 5th day, when an extensive increase in the IDO activity started (Fig. 1 and Table 1), the prolonged mononuclear response was the dominant feature of the tissue reaction. On the 11th day, when the enzyme activity reached its peak, the peribronchial and perivascular infiltrations were marked and almost totally lymphocytic in appearance. On the 14th day, when the enzyme activity had started to decrease, an excessive infiltration of predominantly mononuclear cells of the plasmocytetype was still observed. These results suggest that the increment in the enzyme activity may be related to the inflammatory response.

When mice were exposed to high dose of virus ($10^{7.5}$ EID_{50}/0.1 ml) and did not recuperate, the time course of the increase of enzyme activity was essentially identical to that seen with a low concentration of virus (Fig. 2). Approximately 100-fold increase in the pulmonary IDO activity occurred on the 9th day after infection. However, antibody formation was slight and EID_{50} in the lung remained at a high level, as reported previously (10). These results suggest that the increment

Table 1. Effect of virus infection on indoleamine 2,3-dioxygenase activity in various organs

Organ	Relative specific activity,* 0 time†	1st day	3rd day	5th day	9th day	11th day	14th day	21st day
Brain	1.00 (0.04 ± 0.02)	0.33	1.14	1.53	0.58	0.43	2.01	0.92
Trachea	1.00 (0.07 ± 0.02)	3.00	8.57	12.53	147.14	99.56	22.47	5.71
Thymus	1.00 (0.17 ± 0.03)	1.36	1.31	1.63	3.04	1.16	2.68	1.79
Lung	1.00 (0.07 ± 0.01)	3.39	2.81	6.13	66.30	120.76	65.03	7.67
Pancreas	1.00 (0.01 ± 0.00)	3.87	3.10	2.33	39.80	17.00	5.32	3.06
Spleen	1.00 (0.02 ± 0.01)	2.91	1.33	1.20	4.77	3.21	4.27	1.89
Intestine	1.00 (0.70 ± 0.31)	0.70	0.70	0.52	0.52	0.30	0.47	0.81
Cecum	1.00 (0.24 ± 0.03)	1.18	2.06	6.65	3.93	6.75	2.37	1.40
Colon	1.00 (1.17 ± 0.05)	0.49	0.94	1.19	0.98	1.87	1.19	1.16
Epididymis	1.00 (162.5 ± 20.3)	1.89	1.08	1.20	1.54	1.89	1.19	1.06
Liver‡	1.00 (13.2 ± 3.42)	3.77	1.97	2.12	2.28	1.23	0.61	1.21

After administration of the virus (10^6 EID_{50}/0.1 ml), six mice were sacrificed at various intervals, and the IDO activity was assayed.
* Relative to the specific activity at 0 time.
† Data in parentheses represent the mean ± SEM of the observed specific activity (nmol/min per mg of protein) at 0 time, for six mice.
‡ The tryptophan-cleaving activity in the liver is due to tryptophan 2,3-dioxygenase (16).

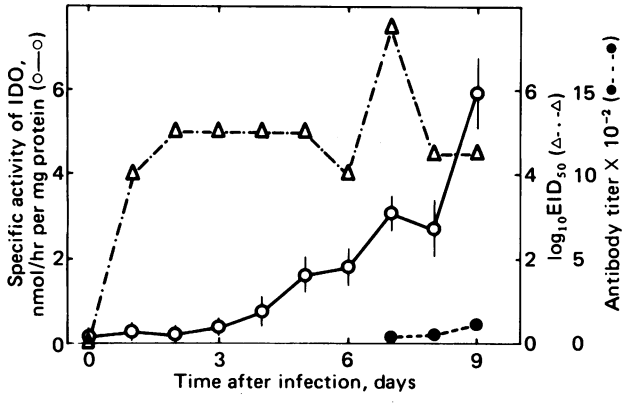

FIG. 2. Virus titer and IDO activity in the lung and serum antibody content at intervals after infection with PR8 virus at $10^{7.5}$ EID_{50}/0.1 ml. Enzyme activity represents the mean ± SD for three mice. Symbols used are as in Fig. 1.

of the enzyme activity during the infection may not be related directly to the antibody formation.

DISCUSSION

In the present study, the enzyme induction appeared to be related to the inflammatory response. Human monocytes and macrophages are capable of generating substantial amounts of the superoxide anion during phagocytosis (18). Serotonin, a substrate of IDO, is one of the substances liberated at inflammatory loci (19). Thus, the enzyme induction may be related to local increase in the production of the superoxide anion or serotonin in the lung due to inflammation, both of which are utilized by IDO (2–5). Paraquat is selectively accumulated by the lung in a variety of animal species (20); when reduced, it reacts avidly with O_2 to form the Paraquat cation and generates O_2^- (21). When Paraquat (0.9 mg per mouse) was administered to mice intraperitoneally, the enzyme activity in the lung was increased approximately 3-fold after about 3 days (data not shown), suggesting that O_2^- is one of the candidates for IDO induction. However, the extent of the enzyme induction was far below the level of maximum induction caused by virus infection or by LPS. By contrast, 5-hydroxy-L-tryptophan administration is known to increase the concentration of its decarboxylated metabolite, serotonin, both in the central nervous system and in various peripheral tissues, and it is generally agreed that the pharmacological effects of 5-hydroxy-L-tryptophan are mediated via the serotonin formed (22). The enzyme induction may not be achieved solely by serotonin or its related substances, because intraperitoneal injection of 5-hydroxy-L-tryptophan was almost ineffective in inducing the enzyme activity. At present it appears that the dramatic induction of IDO observed in the mouse lung after virus infection or intraperitoneal injection of LPS (8) may be explained only partially by the increased production of the superoxide anion as well as other factors, including serotonin.

When lymphocytes are stimulated by influenza virus they produce large quantities of interferon (23). The time course of the increase in the pulmonary IDO activity during virus infection was quite different from that of interferon induction reported by other investigators (10). Interferon induction has been reported to follow the rise in virus titer quite closely. Preliminary experiments in our laboratory indicated that poly(I)·poly(C), a potent inducer of interferon (24), induced the pulmonary IDO activity. However, the time course of the interferon induction by virus infection, poly(I)·poly(C), or LPS (25) always preceded that of the induction of IDO activity. These results suggest that interferon and IDO are different entities but may possibly be induced by a certain common mechanism.

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