STUDIES OF THE IMMUNE RESPONSE IN SENDAI VIRUS INFECTION OF MICE

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PLATES XIX AND XX

IN a previous paper it was shown that Sendai virus infection of mice could be used for the study of the pathogenesis of viral infections of respiratory mucous membranes (Robinson, Cureton and Heath, 1968). In particular, it was possible to use this model system to correlate growth and eradication of virus with certain defence factors such as the inflammatory changes, interferon and antibody production.

In this paper we describe further studies of the immune response that develops in mice infected with Sendai virus.

MATERIALS AND METHODS

Infection of mice. Swiss albino mice of 20–25 g were infected by intranasal inoculation of 10^5 EID₅₀ of Sendai virus.

The procedures used for obtaining specimens from these mice, for the assay of virus in monkey-kidney tissue cultures and for haemagglutination inhibition (HI) tests for antibody have been described previously (Robinson *et al.*, 1968). Virus assays were carried out on pooled lung suspensions obtained from groups of three mice, and antibody titrations were done on individual specimens of serum and bronchial washings or on pools as indicated in the text.

Bronchial secretions. Blood-free samples of the bronchial secretions were obtained by gently washing the lungs of exsanguinated mice with phosphate-buffered saline. The washing procedure was carried out with a small intravenous cannula tied into the trachea and connected to a Mantoux syringe containing 0.5 ml of saline. At least 0.25 ml of bronchial washing was recovered, and this was centrifuged and the supernatant stored at -20°C . Washings were concentrated approximately 5-fold by dialysis against Carbowax.

Histology. Tissue for histology was fixed in cold absolute alcohol and embedded in paraffin wax (Sainte-Marie, 1962). Vertical serial sections of one lung, from each of three mice killed daily, were mounted on a single slide. The reagents used for immunofluorescent staining were a rabbit anti-mouse-immunoglobulin antiserum, kindly provided by Dr G. Torrigiani of the Department of Immunology, Middlesex Hospital, and a fluorescein-conjugated dog anti-rabbit- γ -globulin antiserum (Fluoroscans, Winthrop Biologicals). The lung tissue was dewaxed in xylol and taken through graded concentrations of ethanol into phosphate-buffered saline, pH 7.4. The sections were covered with the anti-mouse-immunoglobulin antiserum for 30 min. and then washed in two changes of buffer, 20 min. each. They were covered with the fluorescein-conjugate for 30 min. and after a further thorough wash were mounted in buffered glycerol.

The fluorescence microscope used was a Leitz Orthoplan, with an Osram 200W mercury vapour burner, BG12 exciter filter and Ilford 102 and 106 gelatin absorption filters. The

Received 29 July 1970; accepted 15 Sept. 1970.

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photographs shown are copies of originals that were taken on Kodak High Speed Ektachrome daylight-type film within a 3-min. exposure time.

The specificity of the test was controlled by (a) including a section from an uninfected lung on each slide, (b) staining one slide from each group with the conjugated antiserum alone, (c) staining another with normal rabbit serum and conjugate, and (d) using a blocking test, which was done by first incubating a slide with a goat anti-mouse- γ -globulin antiserum and then carrying out the full immunofluorescent staining procedure described above.

Immuno-electrophoresis of the rabbit and goat anti-mouse-immunoglobulin antisera showed that both reacted mainly with mouse IgG. There was some cross-reactivity with other immunoglobulin classes, but not with other serum proteins.

Parallel sections of the lungs were also stained with haematoxylin and eosin and by a modified Unna-Pappenheim method.

Electrophoretic procedures. The bronchial washings were examined by immuno-electrophoresis, with an antiserum to whole mouse serum (Hyland Laboratories), by the method of Scheidegger (1955).

TABLE I
Virus growth and antibody production in serum and bronchial washings of Sendai virus-infected mice

Day of infection	Virus titre in lungs (log ₁₀ TCD ₅₀ per ml)	Geometric mean HI antibody titres* in	
		serum	bronchial washings
0	...	6.5	<1
1	<1	...	<1
2	<1	4.0	<1
3	3.2	...	<1
4	4.5	4.9	<1
5	3.5	...	<1
6	3.5	8.0	<1
7	3.5	...	<1
8	1.5	9.8	1.25
9	<1	...	1.25
12	<1	104.0	4.92
21	<1	158.0	1.0

* Calculated from assays performed on three separate specimens.

RESULTS

A batch of 200 mice was infected by intranasal inoculation of Sendai virus. At various intervals after infection, groups of nine mice were killed by exsanguination under ether anaesthesia and observations made on (1) virus growth in the lungs, (2) HI antibody content of serum and bronchial washings, (3) the presence of immunoglobulins and other proteins in the bronchial washings, and (4) histological changes in the lungs, including the appearance of immunoglobulin-containing cells.

Virus growth and antibody production

The relation between the growth of virus in the lungs and the appearance of antibodies in serum and bronchial secretions is shown in table I.

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FIG. 1.—Immuno-electrophoresis of bronchial secretions before and after Sendai virus infection. The anode is to the right and all the troughs contain anti-whole-mouse-serum antiserum. The wells contain, from above downwards, normal mouse serum and then bronchial washings taken before infection and 6, 12 and 33 days after infection.

FIG. 2.—Mouse lung before infection. A single immunoglobulin-containing cell is visible in the bronchial submucosa. Immunofluorescence photomicrograph (IF). $\times 400$.

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FIG. 3.—Lung 2 days after infection. There is an increased number of immunoglobulin-containing cells in the peribronchial and perivascular spaces. IF. $\times 400$.

FIG. 4.—Lung 5 days after infection. A further increase of immunoglobulin-containing cells is present, surrounding a blood vessel. IF. $\times 400$.

FIG. 5.—Lung 9 days after infection, showing aggregations of lymphocyte-like immunoglobulin cells in the submucosa. IF. $\times 400$.

Virus titres reached their peak by the 4th day of the infection and then declined. Virus was not detectable in the lungs after the 8th day. Before infection, a low titre of HI antibody was present in the serum, possibly due to past infection. A small increase in the antibody level was evident by the 6th and 8th days of the infection and an appreciable increase by the 12th and 21st days. The pattern of appearance of antibody in the bronchial washings was very similar, with little or no antibody detectable before the 8th day of infection.

These serological studies clearly showed that there was no significant rise in antibody levels in either serum or bronchial washings until the 6th–8th day of the infection, that is, 2–4 days after virus titres in the lungs had begun to decline.

TABLE II

Relation between antibody production and appearance of immunoglobulins and other proteins in bronchial secretions of Sendai virus-infected mice

Day of infection	HI antibody titres* in		Proteins in bronchial washings†			
	serum	bronchial washings	Globulins			Albumin
			γ	β	α	
0	4	1	+	—	—	++
6	8	1	++++	+	+++	++++
12	64	16	+++	+	++	+++
33	256	8	++	—	+	+++

* Assays carried out on pools of serum obtained from six mice and on pools of bronchial washings obtained from 25 mice.

† — to ++++ = Relative amounts of protein as demonstrated by immuno-electrophoresis.

Immunochemical studies

Sendai virus was inoculated intranasally into a further batch of mice. Bronchial washings were collected from groups of 25 mice killed immediately before or at intervals of 6, 12 and 33 days after inoculation. The washings from each group were pooled and examined by immuno-electrophoresis. Serum samples were also taken at these times for antibody assay.

The results of this experiment are shown in table II and fig. 1. Before infection, only albumin and a small amount of γ -globulin were present in the bronchial secretions. By day 6, increased amounts of protein were present, particularly γ -globulin and albumin. α - and β -Globulins were now detectable and there were several arcs present in the γ region. These alterations were almost certainly due to increased tissue permeability associated with the underlying inflammatory changes in the lungs (*vide infra*). It is of interest that there was no significant increase in the amounts of specific antibody in either the bronchial washings or serum during this early stage of the infection.

Essentially the same electrophoretic pattern for the bronchial secretions

was obtained on *day 12*, apart from the disappearance of a β -globulin and one of the γ arcs. By *day 33*, the pattern was similar to that seen in uninfected mice, although there was an α -globulin arc and there was still some increased electrophoretic heterogeneity of both the γ -globulin and albumin. Significant increases in the levels of specific antibody were detected in both bronchial washings and serum on days 12 and 33.

The γ -globulins that appeared in the secretions at the time of the initial inflammatory response were not associated with detectable specific antibody, and it is unlikely that they played an important role in the virus eradication process. The γ -globulins detected in the 12-day and 33-day secretions were associated with specific antibody activity, but they probably appeared too late to influence the course of the infection.

Histological changes

Examination of the sections stained with haematoxylin and eosin showed that damage to the bronchial mucous membrane was associated with marked inflammatory changes. There was an early transitory polymorphonuclear leucocyte response followed by a more prolonged and intense mononuclear cell response. The changes found were similar to those described in detail by Robinson *et al.* (1968).

The lungs were also examined for the presence of immunoglobulin-containing cells by the indirect fluorescence method. A few such cells were found in the bronchial submucosa of normal lungs taken before infection (fig. 2). Of particular interest was the finding, as early as the *2nd day*, of a marked increase in the number of these immunoglobulin-containing cells, mainly within the perivascular and peribronchial oedema fluid (fig. 3). A further substantial increase in their number had occurred by *day 5*, when many thousands were present in each section compared with less than a hundred before infection (fig. 4). By *day 9*, the cells in the perivascular and peribronchial areas were beginning to organise into lymphoid-like aggregations (fig. 5), and small collections were still present *49 days* after infection.

The most pertinent fact revealed by these histological studies was that immunoglobulin-containing cells made their appearance in the infected tissues several days before the occurrence of rises in free antibody levels.

DISCUSSION

Sendai virus infection of mice provides a useful model for studying simple viral respiratory diseases, since the infection is generally confined to the mucous membranes, and the mice recover. Recovery from viral respiratory infections is generally a remarkably consistent and efficient process, but how it is effected is still not clearly understood. It is generally held that non-specific factors such as inflammation and interferon production are mainly concerned, and it is possible that immune processes play some part.

The immunochemical studies described in this paper gave results which are essentially similar to those obtained in man by Artenstein, Bellanti and

Buescher (1964) and Alford *et al.* (1967). The early inflammatory changes led to the transfer of appreciable amounts of serum protein, including immunoglobulins, into the bronchial secretions. These immunoglobulins could play a part in the termination of the infection only if the serum contained high concentrations of antibody dating from a previous infection. When the inflammatory changes had subsided the secretions contained an increased amount of immunoglobulin associated with specific antibody activity. This is probably locally produced IgA antibody which, although important in preventing re-infection (Smith, Bellanti and Chanock, 1967), could have had little effect on the inducing virus, because of the delay in its appearance.

The failure to detect increased amounts of antibodies in either serum or bronchial secretions until the 2nd wk of the infection confirms results obtained in previous studies (Robinson *et al.*, 1968; Robinson, Cureton and Heath, 1969), and these findings seem to exclude the role of immune processes in the termination of primary virus infections. It was, therefore, of interest to find an increased number of immunoglobulin-containing cells in the infected tissues as early as the 2nd day, and a further increase by the 5th day. At this time, one can only speculate on the function of these cells, particularly as it has yet to be shown that they are actually producing specific antiviral antibody. It is possible that they are secreting small but effective amounts of antibody into their immediate environment, undetected in the present study because of fixation of antibody by excess virus or insensitivity of the tests. Alternatively, the immunoglobulin-containing cells may themselves be capable of directly inactivating newly formed virus. In either case this could be an example of extravascular immunity, as postulated by Hall (1969).

Further work is clearly required to elucidate the mechanisms and role of immune processes in respiratory virus infections. As mentioned above, it will be important to find out whether the antibody in the fluorescent antibody-staining cells, particularly those that appear early in the infection, is specific for the infecting virus, and to establish the class of immunoglobulins being formed by these cells.

SUMMARY

A series of complex immunological changes occur in the lungs of mice infected with Sendai virus. Early in the infection a rapid increase occurs in the number of immunoglobulin-containing cells in the vicinity of infected mucosal cells. Qualitative and quantitative changes occur in the immunoglobulins and other serum proteins in the bronchial secretions at the time of the inflammatory response. High immunoglobulin levels in the bronchial secretions persist long after the inflammatory changes have subsided. The concentrations of detectable specific antibody do not increase until after the commencement of the viral eradication process.

The possible significance of these changes in the termination of primary virus infections is discussed.

We would like to thank Miss G. V. Martin, Mr J. W. Miller and Mrs S. J. Coward for their excellent technical assistance.

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