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BRIEF COMMUNICATIONS

Studies on the Immune Response and Pathogenesis of Sendai Virus Infection of Mice

II. THE IMMUNOGLOBULIN CLASS OF PLASMA CELLS IN THE BRONCHIAL SUB-MUCOSA

G. BLANDFORD * AND R. B. HEATH †

** Gage Research Institute, Division of Clinical Immunology, Toronto Western Hospital and Departments of Medicine and Pathology, University of Toronto, Canada, and † Department of Virology, St. Bartholomew's Hospital, London E.C.*1.

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Summary. The classes of immunoglobulin-containing cells in the lungs of mice after primary infection with Sendai virus were studied using an indirect immunofluorescence technique. It was found that during the first 5 days of the infection there was a considerable increase in all classes of immunoglobulin-containing cells with the IgA-containing cells retaining their pre-infection predominance. By the 6th day of the infection both IgG and IgA cells were present in equal numbers. This 1:1 ratio persisted for nearly 30 days and was followed by a gradual reversion to IgA excess. IgM-containing cells represented only 1/15–1/30 of all immunoglobulin-containing cells at any time during the infection.

INTRODUCTION

Previous studies of the non-lethal infection of mouse lung with Sendai virus suggested that this mild infection was terminated mainly by immune responses (Robinson, Cureton and Heath, 1968, 1969). It was subsequently shown that immunoglobulin-containing cells appear early in the perivascular and peribronchial areas of the lungs of these mice (Blandford, Cureton and Heath, 1971) and that specific antibody to the infecting virus was detectable in the lung tissues in the presence of free viral antigen by the third day of the infection (Blandford and Heath, 1972). Free antibody detected in secretions at the 7th–8th post-infection day was IgG and IgA, but at 33 days was virtually all IgA (Blandford, 1970). The present paper is concerned with studies of the immunoglobulin class of the cells which appear in the cellular infiltrate in the lungs and with their place in the local immune response.

Correspondence: Dr Gerald Blandford, Gage Research Institute, 223 College Street, Toronto, Ontario M5T 1R4, Canada.

MATERIALS AND METHODS

The animal model

A primary non-lethal parainfluenza 1 (Sendai) virus infection of mice was used. The procedures for intranasal infection and obtaining specimens have been previously described (Robinson *et al.*, 1968; Blandford *et al.*, 1971). In four separate experiments, using from 150–300 mice for each study, tissues were taken before and at varying intervals after infection. Uninfected animals kept in identical environmental conditions were used as controls.

Histological techniques

The lungs and spleens of three exsanguinated animals on each experimental day were dissected and fixed in cold alcohol and mounted in paraffin wax (Sainte-Marie, 1962). Paraffin blocks were prepared with three lungs and three spleens from the day of the experiment and one lung and one spleen from an uninfected control animal. Consecutive parallel sections were mounted in pairs on 3 inches × 1 inch glass slides and stained as indicated below.

Antisera

Monospecific rabbit antisera to mouse IgM, IgG and IgA were kindly provided by Dr B. A. Askonas, National Institute for Medical Research, Mill Hill, London. The anti-IgG and anti-IgA antisera were mixtures of antisera to IgG1 and IgG2 and IgA2 and IgA2 myeloma proteins respectively. The antisera did not cross-react on Ouchterlony analysis. A fluorescein-conjugated sheep anti-rabbit γ-globulin antiserum (SARG) was used throughout and has been previously described (Blandford and Heath, 1972).

Immunofluorescent staining

A standard immunofluoresence technique was used. Four consecutive multiple block sections from each experimental day were examined (two slides). The groups of tissue sections were separated from each other, as previously described (Blandford and Heath, 1972) and were covered for 30 minutes with anti-IgG and anti-IgA on one slide, and anti-IgM and phosphate-buffered saline, pH 7·4, on a second slide. The slides were then washed in two changes of buffer for 1 hour and the whole slide covered with SARG for 30 minutes, and washed again before mounting. Slides were examined by dark ground microscopy with u.v. light (Blandford and Heath, 1972).

The conjugate alone did not stain the sections. As only small quantities of monospecific rabbit antisera were available and an indirect technique was used, blocking controls were not possible. Instead a sequential immunofluorescent technique was used as a further check for specificity of antisera (Nash, Crabbe and Heremans, 1969). Sections were stained in sequence with anti-IgM, anti-IgA and anti-IgG and photographed at each step. Although there was some build-up of non-specific fluorescence, it was quite clear that different cell populations were stained with each reagent.

Cell counting

Because of the considerable variation in the number of immunoglobulin-containing cells in the lungs at various times after the infection, the following procedure was adopted. Up to the 5th day and from the 21st day after infection, all peribronchial and perivascular

positive-staining cells on complete sections from each of three mice, on each day of each experiment were counted and averaged. This usually involved 200 high power fields (magnification ×400). Between the 7th and 15th days after infection too many cells stained for IgG and IgA for counting of complete sections to be practical. The operational procedure chosen for counting at this time was to locate specific areas and structures on the parallel sections and count all the positive cells of each class in their immediate vicinity in fifty high power fields.

With the spleens, comparative areas of sections from three mice on each day were counted from fifty high power fields and were averaged.

Bronchial lymph nodes were found infrequently. They were small structures and it was uncommon for more than ten to fifteen immunoglobulin-containing cells to be detected in them. The total number of cells in each class were counted.

RESULTS

The results obtained in the first experiment are presented in the table. This shows the number of cells of each individual immunoglobulin class that were present in lung sections of mice at varying intervals after infection with Sendai virus. It can be seen that there was an increase in numbers of cells of all immunoglobulin classes beginning at day 2, and most marked between days 6–15. At the end of all experiments there were always more cells of all classes present than before infection or than in control, uninfected animals.

TABLE 1

COUNTS OF IMMUNOGLOBULIN-CONTAINING CELLS IN THE LUNGS OF MICE AFTER A PRIMARY SENDAI VIRUS INFECTION

Day	Number of IgA-containing cells	Number of IgG-containing cells	Number of IgM-containing cells
Uninfected			
0	298	67	38
70	381	130	51
After infection			
1	261	83	44
2	1292	317	108
3	849	224	96
4	918	379	116
5	1384	1012	176
6	1201 *	1466	297
7	1135	1271	277
8	1056	1256	194
9	1183	1162	180
12	1080	1172	206
15	1177	1138	156
21	682	699	175
27	818	760	91
35	609	402	119
49	478	304	109
70	511	360	93

* Counts underlined are per fifty high power fields (magnification × 400). All other counts (i.e. on days 0–5 and 21–70) are per whole lung section (average of 200 high power fields). Counts are averaged from three animals on each day.

Up to and including day 5, IgA cells predominated. After this time IgA and IgG cells were present in roughly equal numbers with a return towards excess IgA cells from day 27. IgM cells were present in relatively small numbers throughout and paralleled the increase seen with IgG cells. Increased numbers of cells affecting IgG more than IgA were also found in uninfected animals at day 70. At the termination of the experiment there were roughly twice as many IgA and IgM cells and three times as many IgG cells than in uninfected control animals at the same time. Essentially similar results were obtained in other experiments.

The figure shows changes in the IgG:IgA cell ratio in the lungs and spleens of mice, averaged from the counts of the four experiments. Before infection, IgA cells predominated in the lungs at a ratio of about 1:4 but from the 6th–26th day the ratio was closer to 1:1. This is in marked contrast to the spleen, where IgG cells were always considerably in excess. It is apparent that there was a greater proportional increase of IgG to IgA cells in response to the infection in both organs. In the lungs the proportional increase of IgG cells persisted at least up to day 70 and it was transient in the spleen between days 5–9. From day 27 onwards, the lungs again showed IgA cells in excess.

Insufficient numerical data was obtained from the sections of the bronchial lymph nodes, to make meaningful day to day comparisons, however, on no occasion were IgA cells equal to or in excess of IgG cells.

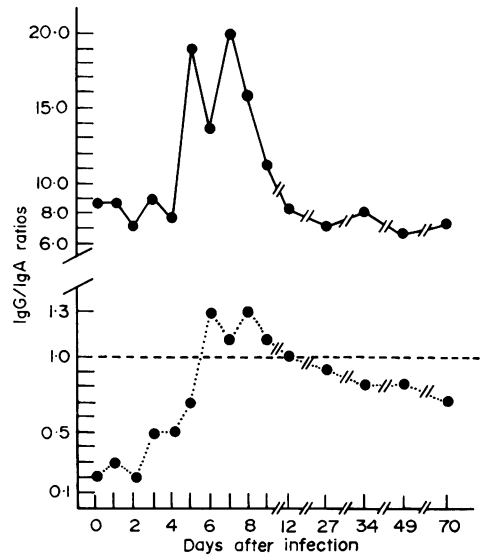

FIG. 1. IgG/IgA ratio in the (· · · ·) lungs and (—) spleens of mice at intervals after a primary Sendai virus infection.

DISCUSSION

Following the damaging immunogenic attack induced by Sendai virus infection the immunoglobulin-secreting cell population beneath the respiratory sub-mucosa increased substantially, as previously shown (Blandford *et al.*, 1971). The pre-infection predominance of IgA cells in the lungs which we observed was expected and has been reported to occur in man and several animal species (Bienenstock and Tomasi, 1969). It had been

expected that IgA cells would preferentially accumulate after infection, but surprisingly, by day 5 there was only a five-fold increase in IgA cells compared with a twenty-fold increase in IgG cells. This trend for the apparent preferential accumulation of IgG cells continued for a further week. Similar findings have been reported following mumps virus infection of the salivary glands of monkeys (Genco, Flanagan and Emmings, 1972).

Previous studies with this model have strongly suggested that the local immune response had a predominant role in the termination of the infection (Robinson *et al.*, 1969; Blandford *et al.*, 1971). From the present work it now seems that both the IgA and IgG mechanisms may be involved, but for the moment it is not possible to state which of these is the most important. This could perhaps be resolved by studying the early appearance of specific IgA and IgG antibodies in the bronchial secretions.

Finally, it must be remembered that although IgM cells and antibody appear to play a relatively insignificant local role at the infected respiratory surface, it is not possible for either to be firmly excluded from playing a part.

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