Analysis of the local and systemic immune responses induced in BALB/c mice by experimental respiratory syncytial virus infection

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Introduction

Since respiratory syncytial (RS) virus was first isolated in 1955, it has become recognized as the single most common cause of morbidity during infancy and early childhood. Although the virus may produce a variety of respiratory diseases, the more severe forms of disease, namely bronchiolitis and bronchopneumonia are largely confined to those infants less than 1 year of age. Development of a safe and efficient means of providing prophylaxis is therefore currently of prime importance. However, past attempts to develop an effective RS virus vaccine have met with little overall success. In a well documented series of clinical trials initiated in the late 1960s, a formalin-inactivated vaccine was used to immunize infants and young children, but was found not to reduce the incidence of subsequent RS virus infection (Chin et al., 1969; Kapikian et al., 1969; Kim et al., 1969). More disturbingly, the vaccine was found to potentiate the development of severe disease among vaccinees upon re-exposure to the virus. Recent reports have suggested that this may have been due to the formalin inactivation (Murphy et al., 1986), which may have destroyed epitopes inducing neutralizing antibodies while leaving intact those epitopes involved in induction of immunopathology. In addition, indications that an immediate hypersensitivity response could contribute to the development of severe disease in the normal population (Welliver et al., 1980, 1981, 1984) have highlighted the need for a greater understanding of the precise role of the host's immune response. A more detailed analysis of the immune response would allow definition of protective and potentially injurious responses to the virus.

Study of the human immune response has proven difficult because severe RS virus disease occurs most frequently in infants less than 1 year old. It has been possible to monitor peripheral antibody and cell-mediated immune responses to infection, but technical difficulties have severely restricted analysis of the responses produced in the respiratory tract, and particularly the lower respiratory tract. The use of suitable animal models, however, provides an alternative means of analysing these immune responses. Cellular responses to RS virus infection in the lungs of both cotton rats and
inbred laboratory mice have been described (Kumagi et al., 1985; Sun et al., 1983a, b; Taylor et al., 1985). A correlation has been found between the decline of virus shedding within the lungs of infected cotton rats and the activation of a pulmonary population of natural killer (NK) cells. In contrast, research involving BALB/c mice has described the development of a primary pulmonary virus-specific, major histocompatibility complex (MHC)-restricted cytotoxic T lymphocyte (CTL) response, which also correlates with the decline of virus infection. In addition, the ability of secondary stimulated CTLs from spleen to resolve persistent infection in immunologically deficient mice has been reported (Cannon et al., 1987). The present study provides a broad comparative analysis of the kinetics of a variety of acute immune responses induced in the lungs of BALB/c mice after experimental RS virus infection. The possible contribution of each response to the control and elimination of virus from the lungs of infected mice is discussed.

Methods

Virus stocks. Stocks of the A2, Long (group A) and 8/60 (group B) strains of RS virus as well as stocks of parainfluenza virus type 3 were grown in monolayers of HEp-2 cells maintained in medium E199 supplemented with 10% (v/v) heat-inactivated foetal calf serum (HIFCS), penicillin, streptomycin and glutamine. The A2 and 8/60 strains of RS virus were originally obtained from Dr E. J. Stott (formerly of the Institute for Research on Animal Diseases, Compton, U.K.); the Long strain of the virus was obtained from the Central Public Health Laboratory Service. Stocks of encephalomyocarditis (EMC) virus were grown in murine L929 fibroblasts. EMC virus was supplied by Dr C. E. Taylor (The Department of Virology, The Royal Victoria Infirmary, Newcastle upon Tyne, U.K.). All virus stocks were stored at -70°C and thawed immediately prior to use.

Infection of mice. Eight to 12 week old (mean age 98 ± 1.5 weeks) specific pathogen-free BALB/c mice (Newcastle colony) were obtained from the Comparative Biology Centre of the University of Newcastle upon Tyne. All mice were inoculated under light ether anaesthesia. Groups of test mice were inoculated with 50 μl volumes of A2 strain RS virus stock, adjusted to contain 3 × 10⁶ p.f.u. of infectious virus per ml of suspension (1.5 × 10⁶ p.f.u. per mouse). Groups of control mice were mock-infected with equivalent volumes of clarified, uninfected HEp-2 cell culture supernatant.

Virus infectivity. Quantification of infectious virus in RS virus stocks as well as in lung homogenates was achieved by virus plaque assay performed as described previously (Toms et al., 1980).

Immunofluorescence staining of lung tissue. Specimens of lung tissue from individual mice were snap-frozen in isopentane, held at -70°C and used to prepare frozen sections. These were stained and examined in accordance with procedures outlined previously (Routledge et al., 1988). Absorbed reagents did not produce non-specific fluorescence when reacted with lung tissue from mock-infected mice or normal uninfected tissue culture specimens.

Histology. Lungs were inflated with formal saline introduced via the trachea. After storage for 48 h in this fixative, tissue was dehydrated by passage through 70%, 90% and ultimately absolute methanol. Alcohol was subsequently displaced with toluene and the tissue was then embedded in paraffin wax. Later, 4 to 6 μm sections were cut, rehydrated, stained with haematoxylin and eosin, mounted and then examined.

Preparation of lung lymphocyte suspensions. In most instances lung tissue was disrupted without prior enzymic digestion by gently rubbing tissue through 106 μm mesh stainless steel sieves. However, preparation of lung lymphocytes for NK cell assays included predigestion of tissue with collagenase. Briefly, minced tissue was continually agitated in 25 mm-HEPES-buffered RPMI 1640 supplemented with 5% (v/v) HIFCS and containing Sigma Type 1 C 0130 collagenase (120 units per pair of lungs). After 90 min incubation residual lung tissue was disaggregated by passage through test sieves. Lymphocytes were ultimately extracted from crude lung cell suspensions by fractionation upon discontinuous sodium metrizoate (11.6% w/v) and dextran (42% w/v) gradients (Nygaard). Lymphocytes recovered from the interfaces of these gradients were washed three times by centrifugation through RPMI 1640 before finally being resuspended in HEPES-buffered RPMI 1640 containing 10% (v/v) HIFCS, 2 mM-glutamine, 100 units/ml penicillin G and 100 μg per ml of streptomycin sulphate.

Preparation of splenic leukocytes. Crude splenic leukocyte suspensions were prepared by passing spleen tissue from individual animals through 106 μm mesh sieves. In proliferation assays erythrocytes were not removed from cell suspensions, otherwise erythrocytes were lysed using Tris-buffered ammonium chloride solution (pH 7.2), and residual viable splenic leukocytes were deposited by centrifugation through a cushion of HIFCS. Splenic leukocytes were again washed by centrifugation through RPMI repeatedly before finally being resuspended in 10% (v/v) HIFCS-RPMI.

Cytotoxicity assays. Cellular cytotoxicity in different effector cell suspensions was assessed by monitoring 51Cr release from pre-labelled target cells in a modification of the assay system adopted by Steele et al. (1973). Briefly, CTL-mediated cytotoxicity assays were performed in triplicate in 96-well microculture trays. In each assay, 5 × 10⁴ Na51CrO4-labelled BALB/c 3T12-3 fibroblasts infected 24 to 96 h previously with various strains of RS virus (m.o.i. 10 p.f.u./cell) were reacted with between 1.5 × 10⁴ and 5 × 10⁴ effector cells (effector: target cell ratios of 3 : 1 to 100 : 1). Parallel control assays were routinely constructed using uninfected 3T12-3 target cells. Assay plates were centrifuged at 150 g for 5 min before incubation in an atmosphere of 5% CO2 for 8 h. After this time 100 μl aliquots of assay supernatants were withdrawn and the quantity of radioactivity was measured in a gamma-counter. The percent specific immune release of radiolabel induced in each instance was defined as [(specimen c.p.m. – spontaneous c.p.m.)/(maximum c.p.m. – spontaneous c.p.m.)] × 100, where maximum c.p.m. is equal to that amount of radioactivity released by targets treated with a 10% (v/v) solution of Decon 90 detergent. The mean spontaneous release of radiolabel from RS virus-infected 3T12-3 target cells was found to be 29.5% ± 6.9% over the 8 h assay period. Indirect membrane immunofluorescence indicated that an average of 67% of the RS virus-infected 3T12-3 target cells used in these assays expressed significant quantities of RS virus antigens detectable with virus-specific polyclonal rabbit antisera. Using murine monoclonal antibodies, 2A strain-infected 3T12-3 fibroblasts were found to express viral F, G, P and 22K products. Assessment of NK cell activity was made using 4 h cytotoxicity assays, employing YAC-1 lymphoma cells as target cells. Construction of these assays was performed as described above. The mean spontaneous release of 51Cr from labelled YAC-1 targets in these assays was found to be only 8.6% ± 0.6%.

Depletion of cytotoxic lymphocytes with anti-Ly2 antibody and complement. Pulmonary lymphocytes (6.2 × 10⁷) were incubated for
60 min at 4 °C in 6 ml of 1/200 monoclonal anti-Ly2 (CD8) antibody (monoclonal antibody CRC 3.155 was kindly supplied by Dr J. H. Robinson of the Department of Anatomy of the University of Newcastle upon Tyne, U.K.). Sensitized cells were washed and resuspended in a 1/10 dilution of rabbit complement (Low-Tox M, Sera-Lab) for 60 min at 37 °C. Residual viable lymphocytes were recovered by fractionation upon discontinuous density gradients, washed and then adjusted to 5 × 10^6 viable cells/ml in 10% (v/v) RPMI 1640. Control cell suspensions consisting of untreated lymphocytes as well as lymphocytes treated with rabbit complement alone were tested in parallel with antibody-depleted lymphocytes in ^51Cr release assays incorporating RS virus-infected and control uninfected 3T12-3 target cells.

Proliferation assays. The proliferation assay employed in this study was a modification of an assay described in detail previously (Scott et al., 1981). In triplicate test assays, 2 × 10^5 viable splenic or pulmonary effector cells (in 200 μl volumes of 10% HIFCS-RPMI) were challenged with multiple dilutions of heat-inactivated RS virus (undiluted, 1:4, 1:16, 1:64) or control HEp-2 (undiluted, 1:4, 1:16) antigens. In parallel control assays effector cells were challenged with phytohaemagglutinin or with medium alone. After fixed periods of incubation, 2 days for mitogen-stimulated cultures, and 3, 4, 5 and 6 days for antigen-stimulated cultures, effector cells were pulse-labelled for 18 h with 0.2 μCi (40 μl) of [3H]thymidine (specific activity 2 Ci/mmol). Cultures were harvested and radio-nucleoside uptake was determined by liquid scintillation counting. Lymphocyte proliferation following challenge with RS virus antigen was expressed as a stimulation index (S.I.) defined as the ratio of geometric mean counts per min from RS virus-challenged cultures, to that from control HEp-2-challenged cultures.

Tracheobronchial lavages. The lower respiratory tract from below the cricoid cartilage was removed from mice at post-mortem. The outer surfaces of the lungs were then rinsed carefully with 5% (v/v) HIFCS-RPMI. For interferon (IFN) assays, washes were prepared by alternately instilling and aspirating 0.3 ml aliquots of 5% (v/v) HIFCS-RPMI into and from the lungs via the trachea of both infected and control mock-infected animals. The washes were clarified by centrifugation at 400 g and then stored at −70 °C. For antibody assays, lavages were prepared by repeatedly lavaging the lungs with 2 ml volumes of cold Hank's balanced salts solution (HBSS), introduced transtracheally. Lavages from groups of three animals were pooled on each occasion and then clarified by centrifugation at 400 g for 5 min. Pooled lavage samples were transiently stored at −40 °C. These specimens were then thawed and supplemented by the addition of bovine serum albumin to a final concentration of 1% (w/v) and then dialysed against Elgastat 2000. Control cell suspensions consisting of untreated lymphocytes as well as lymphocytes treated with rabbit complement alone were tested in parallel with antibody-depleted lymphocytes in ^51Cr release assays incorporating RS virus-infected and control uninfected 3T12-3 target cells.

Interferon assays. IFN was assayed using a modification of the dye uptake microtechnique described by Finter (1969). Tracheobronchial lavages were titrated in parallel with a National Institute for Biological Standards and Control (NIBSC) standard mouse interferon preparation (NIBSC standard a 70/332). Triplicate monolayers of murine L929 cells grown in 96-well microculture plates were washed, overlaid with 150 μl aliquots of the various standard dilutions, lavage sample dilutions or medium alone (cell and virus controls) and incubated for 18 h at 37 °C. Test and virus control monolayers were subsequently challenged with EMC virus. Cell controls were incubated with medium alone. After 48 h incubation, neutral red uptake was measured spectrophotometrically at 540 nm. Titres of individual tracheobronchial lavage specimens were defined in relation to the standard, thus allowing titres to be expressed in terms of international units of interferon per ml (i.u. IFN/ml). The lower mean sensitivity of the assay was 5.0 i.u. IFN/ml as defined by repeated titration of the standard.

Antibody assays. RS virus-specific IgG and IgA antibody titres of plasma, lung lavage and nasal wash samples were determined by modifications of the membrane immunofluorescent antibody test described previously (Scott et al., 1976). Assays employed absorbed goat anti-mouse IgG and IgA Fc-specific fluorescein isothiocyanate conjugates (Cappel Laboratories).

Results

Virus infection of BALB/c mice

Infectious virus was isolated from the lungs of experimentally infected mice between day 1 and day 7 post-infection with a peak at day 4 (Fig. 1). In addition, non-symmetrical antigenic foci of RS virus infection were also identified in cryostat sections of lung tissue collected from animals sacrificed between these days, but not in any of the controls. Both the number and extent of the foci of infection found in infected mouse lung sections increased progressively from day 2 post-infection to maximum levels by days 4 and 5 post-infection and then declined (Fig. 2). All foci of infection were found to be restricted to the alveolar regions of the lungs and no significant indication of bronchiolar epithelial cell infection was observed in any of the sections examined.

Fig. 1. Replication of RS virus in the lungs of Newcastle BALB/c mice. Individual points represent the mean quantity of infectious virus (± one S.D.) recovered per g of lung tissue from groups of three mice.
Fig. 2. Progression of infection within the lungs of A2 strain RS virus-infected mice. Immunofluorescence revealed a general increase in the size of antigenic foci of RS virus infection from day 2 post-infection (a) through days 4 and 5 (b, c) prior to general decline by day 6 (d). Bar marker represents 10 μm.

Histology of infected lung

Transverse paraffin wax sections of lung tissue were examined from groups of four BALB/c mice (three infected and one mock-infected) killed on days 3, 5, 7 and 9 post-intranasal infection. All sections were read blind. The pathological changes that occurred within the lungs of experimentally infected mice were distinct but minor.

In general, pulmonary RS virus infection induced a mild cellular infiltration of the alveolar walls. The cellular infiltrate consisted predominantly of mononuclear cells although occasional polymorphs were seen in close association with the alveolar walls. No peribronchiolar or perivascular lymphocyte cuffing was observed in any tissue section. An irregular thickening of the alveolar walls was also associated with infection. Occasionally,
Immunity to RS virus in BALB/c mice

Fig. 3. Lysis of YAC-1 lymphoma target cells induced by lymphocyte suspensions prepared from the lungs (O), peripheral blood (A) and spleens (r) of BALB/c mice during the immediate 7 day period following RS virus infection. Lung and peripheral blood lymphocytes were pooled from groups of three to six mice and splenic lymphocytes were tested from three individual mice. The mean lysis is expressed ± 1 s.d. The degree of lysis induced by lung (O), peripheral blood (A) and splenic lymphocytes (r) from control uninfected animals is plotted at day 0.

Fig. 4. Lung lymphocyte (CTL)-mediated lysis of RS virus (A2 strain)-infected (O) and control uninfected (●) 3T12-3 BALB/c fibroblasts during the 12 day period following intranasal infection.

Fig. 5. MHC restriction and virus strain specificity of cytotoxic lung lymphocytes collected from BALB/c mice 7 days after infection with the A2 strain of RS virus. (a) They did not lyse control uninfected (●) or parainfluenza virus type 3-infected (A) BALB/c fibroblasts or either A2 strain-infected (●) or control uninfected allogeneic C3H-L929 target cells (■). (b) These cells actively lysed BALB/c fibroblasts infected with the A2 (O), Long (●) and 8/60 (●) strains of the virus.

cells immediately adjacent to the lumina of the alveoli were enlarged as a result of cytoplasmic distension. Enlarged cells occasionally were found in the lumina of the alveoli. Changes were first detectable at 3 days and most apparent 7 days after infection but had essentially resolved by day 9.

The cellular immune response to infection

(i) Natural killer cell response
The level of target cell lysis induced by pulmonary effector cells recovered from enzymically pre-digested lung tissue rose rapidly from day 1 post-infection (% lysis, 22-1) to reach maximum levels (% lysis, 42-8) 3 days after infection, before declining to pre-infection levels by day 7 (Fig. 3). Effector cells from 3 day mock-infected mice and uninfected mice induced only low but comparable levels of YAC-1 target cell lysis (% lysis, 2-6 and 4-7 respectively). In contrast to the situation observed in the lungs of infected mice, the mean level of YAC-1 target cell lysis elicited by splenic leukocytes increased only marginally above basal levels on day 1 post-infection before subsequently declining. Additionally, peripheral blood lymphocytes from infected mice induced levels of YAC-1 target cell lysis similar to lysis by equivalent populations of cells recovered from mock-infected and normal control mice.

(ii) The cytotoxic T cell response
Lymphocytes recovered from the lungs of mice between 6 and 12 days post-infection were found specifically to lyse RS virus-infected, but not uninfected 3T12-3 target cells, with maximum target cell lysis (% lysis, 49-0) being recorded on day 7 (Fig. 4). In contrast, lymphocytes recovered from the lungs of infected mice between 2 and 5 days post-infection failed to lyse either RS virus-infected or control 3T12-3 target cells. In a control experiment, lymphocytes recovered from the lungs of mice mock-infected with HEp-2 tissue culture supernatant failed to develop cytotoxic activity over the 12 day period following infection.

Although infection induced a strong pulmonary cytotoxic response, it did not induce a significant response in the spleens of BALB/c mice, groups of which were examined between 1 and 9 days after infection.

The character of the effector cells mediating the specific local cytotoxic response was established in a series of experiments involving lung lymphocytes ob-
tained on day 7 post-infection (Fig. 5). Firstly, it was found that cytotoxic activity was both RS virus-specific and MHC-restricted. Effector cells actively lysed A2 strain-infected 3T12-3 (H2d) targets but did not lyse parainfluenza virus type 3-infected 3T12-3 targets or A2 strain RS virus-infected allogeneic L929 (H2k) targets (Fig. 5a). Secondly, it was found that expression of cytotoxic activity could be inhibited by preincubating effectors with Ly2 (CD8)-specific monoclonal antibody alone prior to assay. Expression of the Ly2 molecule by the active cytotoxic cells was confirmed by elimination of Ly2⁺ cells from pulmonary effector cell populations by treatment with specific monoclonal antibody plus complement. In such experiments the proportion of RS virus-infected target cell lysis induced by pulmonary lymphocytes was reduced from 33-3% to 4.7% by treatment with Ly2-specific antibody plus complement. Complement alone did not significantly reduce virus-infected target cell lysis (% lysis, 32.4). Finally, it was found that these primary RS virus-specific cytotoxic cells were able to recognize and lyse histocompatible target cells infected with strains of RS virus representative of both major antigenic subgroups of the human virus (Fig. 5b). 3T12-3 target cells infected with Long (group A) or 8/60 (group B) strains of the virus were lysed by effectors recovered from A2 strain-infected mice with an efficiency equal or greater to that of targets infected with homologous A2 strain virus.

(iii) The lymphoproliferative response
The peak RS virus antigen-induced proliferative responses produced by pulmonary lymphocytes recovered from mice between days 1 and 24 post-infection are presented in Fig. 6(a). Significant virus-specific proliferation was observed only in those cultures prepared from mice sacrificed 3 days after infection. In this instance, significant responses were detected in cultures pulse-labelled both 5 and 6 days after in vitro challenge. In each instance control antigen-stimulated cultures showed proliferation which was comparable to unstimulated cultures. Splenocytes from three of six experimentally infected animals responded significantly to challenge with crude RS virus antigen in vitro by 5 to 6 days post-infection (Fig. 6b). In contrast to the response observed within the lungs, the mean magnitude as well as the frequency of such splenic responses increased to reach maximum levels 24 to 34 days after infection. Despite a gradual decline in the mean level of responsiveness shown by cultures of splenocytes from infected mice beyond this time, virus-specific lymphocyte proliferation was still evident in cultures from half of those mice challenged 56 days after infection.
The interferon and virus-specific immunoglobulin responses

(i) Interferon
Mean interferon levels in tracheobronchial washes rose rapidly following infection and remained elevated between day 1 and day 6 post-infection, before declining thereafter (Fig. 7). However, considerable variation was found in the level of interferon present in washes collected from individual animals on the same day after infection and not all animals appeared to produce detectable levels of interferon. In contrast, the level of interferon in the lungs of normal control mice was found to be uniformly below the mean level of sensitivity of the bioassay (5-0 i.u. IFN/ml).

(ii) The immunoglobulin response
A virus-specific serum IgG antibody response was initially detectable at only 3 days post-infection when seroconversion was observed in two of the three mice examined (Fig. 8). Beyond day 3, mean virus-specific serum IgG levels continued to rise steadily with a plateau declined progressively beyond this time with a greater first class of virus-specific immunoglobulin to be seroconversion was observed in two of the three mice after infection when two of three mice examined proved seropositive. Following primary infection IgG was the first class of virus-specific immunoglobulin to be detected free in concentrated lung lavages. However, it was not detected until day 10 post-infection. A rapid eightfold increase in the level of virus-specific IgG in these specimens was recorded between day 10 and day 24. Free virus-specific IgA was not detected in the lung until day 24 post-infection. Although pooled concentrated nasal washes were also examined at intervals after primary infection, neither virus-specific IgG nor IgA could be detected in these samples (data not shown).

Discussion

In the search for an adequate animal model of RS virus disease, numerous studies have examined the ability of the virus to infect both the upper and lower respiratory tract of a variety of different species. Such research has led to the emergence of two species of rodents, namely cotton rats and more recently laboratory mice, as the primary small laboratory animal models of RS virus infection (Dreizen et al., 1971; Prince et al., 1978; Taylor et al., 1984b). In this report the grade of infection produced in the lungs of the Newcastle colony of BALB/c mice proved to be significantly lower than that described in previous studies (Taylor et al., 1984b, 1985; Graham et al., 1988). This is highlighted by both the lower yield and the duration of virus shedding, as well as by the sparse distribution of viral antigen and mild histopathological lesions which were apparent within the lungs of our animals. It is becoming evident that a number of factors determine the extent of infection produced in BALB/c mice. We have recently compared pulmonary infection in three independent colonies of BALB/c mice and observed reproducible 15-fold variations in the maximum pulmonary yield of virus recovered from these animals (unpublished observations). Genetic differences independent of haplotype may at least in part account for the different susceptibilities of independently maintained colonies of BALB/c mice to RS virus infection. Recent research has also shown that virus culture history, inoculum titre and host age significantly affect the extent of infection and subsequent development of illness in BALB/c mice (Taylor et al., 1984b; Graham et al., 1988).

The first host immune response to be elicited in the lungs of Newcastle BALB/c mice experimentally infected with RS virus was rapid IFN production. The type of IFN found in lung lavages was not defined in the present study. However, preliminary findings indicated that this IFN was stable at low pH (2.0) (unpublished observations). The level of IFN found in individual washes at any one time after infection was found to vary considerably. This may be taken to suggest that animals differ significantly in their ability to produce IFN in response to RS virus infection. However, we were unable to exclude the possibility that such variability may have reflected lung lavage sampling variability. Whether local IFN production exerted any effect upon virus shedding in the lungs was unclear. It is notable that although mean levels of IFN in lung washes rose rapidly after infection, virus replication continued to progress between days 1 and 4 post-infection during which time IFN levels were highest. Currently no data other than those presented in the present report have been published on IFN in RS virus infection in vivo using the BALB/c mouse model. However, recent research in vitro has shown that RS virus infection of BALB/c mouse embryo cells as well as C3H mouse-derived L929 cells induces marked IFN production (Hanada et al., 1986). Furthermore, infection of BALB/c mouse embryo cultures was shown to be limited by this response.

Rapid IFN production in the lungs of BALB/c mice following experimental RS virus infection was accompanied by rapid activation of a population of cytotoxic lymphocytes capable of lysing NK cell-susceptible YAC-1 lymphoma cells. Such activation was confined to the lungs and, although a marginal rise in NK cell activity was observed in the spleens of infected
animals 1 day after infection, this did not persist. In addition, no rise in peripheral blood lymphocyte (PBL) NK cell activity was recorded throughout the immediate 7 day period following infection. The precise phenotype of the activated pulmonary NK-like cytotoxic cells was not clearly defined in the present study, but is currently under investigation. However, the predominance of Thy 1.2-negative and surface immunoglobulin-negative cells, as well as Thy 1.2-positive and CD4- and CD8-negative lymphocytes in bronchoalveolar lavage fluids collected from BALB/c mice infected 3 days previously with RS virus has been described (Openshaw, 1989). Whether the rapid rise in pulmonary NK cell cytotoxicity observed in the lungs of our animals was modulated locally by virus-induced IFN is at present unclear. It has been shown that, although IFN may activate and enhance NK cell activity, activation may also be achieved directly by certain virus glycoproteins including the HN products of parainfluenza virus type 1 and mumps virus (Horfast et al., 1980; Alsheikly et al., 1983). Thus it is possible that IFN-dependent and -independent mechanisms could contribute to activation of the NK-like cells found in the lungs of our infected animals.

Research performed using cotton rats has described an inverse correlation between the development of an active population of cytotoxic NK cell-like lymphocytes in the lungs of RS virus-infected animals and replication of the virus. Furthermore, it has been shown that congenitally athymic C3H/HeN (nu+/nu ÷) mice, despite their T cell deficiency, resolve experimental RS virus infection with the Long strain within 7 days (Wyde et al., 1983). It has been proposed that the high endogenous NK cell activity exhibited by such animals could aid resolution of infection. In the present study however, virus titres in the lungs of our infected animals was modulated locally by virus-induced IFN, while the presence of the congenital athymic trait was crucial for the animals to be unable to resolve experimental RS virus infection (Taylor et al., 1985; Cannon et al., 1987). Adoptive transfer of secondary virus-specific CTLs to these animals has also been shown to clear the virus rapidly from the lungs of these otherwise persistently infected animals (Cannon et al., 1987).

Studies of the cellular immune response to RS virus by infected human infants have of necessity been primarily restricted to the systemic lymphocyte transformation response. These studies have consistently described convalescent stage PBL responsiveness to in vitro RS virus challenge (Scott et al., 1978; Welliver et al., 1979; Cranage & Gardner, 1980; Mito et al., 1984). However, there has been some disagreement over whether this responsiveness may be detected during the acute phase of illness. Using the BALB/c mouse model we found that splenocytes from 50% of experimentally infected mice during the acute phase and 80-90% of mice during convalescence responded to challenge with RS virus antigen in vitro. In addition, it was found that the RS virus-specific CTL response observed in the lungs of our mice was preceded by a rapid but transient virus-specific lymphocyte transformation response. Cells responding to in vitro challenge with virus were recovered from the lungs on day 3 post-infection but were absent by day 7 and at all further times. Induction of a similar rapid local response has also been described in the lungs of influenza A virus-infected C57BL/6 mice within 3 days of infection (Shapira-Nahor & Zakay-Rones, 1985), although responsiveness persisted for a further 9 days in this instance.

Cellular immune responses (NK cell, CTL and lymphocyte transformation) were all detected within the lungs of our experimentally infected mice within 6 days of infection. However, virus-specific IgG antibody could not be detected in pooled, concentrated lung lavages until day 10 and IgA could not be detected until day 34. The successive appearance of virus-specific IgG and much later IgA in the lungs of RS virus-infected mice is comparable with that observed in the lungs of para-influenza virus type 1-infected mice (Charlton & Blandford, 1977). As RS virus-specific IgG could not be detected in the lungs until 10 days after infection, this antibody is unlikely to contribute to recovery from a primary pulmonary infection. However, the possibility that low levels of antibody may have been present in the lungs before day 10 could not be completely excluded. It is known that very low levels of antibody given passively to the respiratory tract of cotton rats effectively reduces

The temporal relationship described between the decline of infection and the induction of pulmonary RS virus-specific CTLs in this study adds further weight to the growing body of evidence indicating that these cells are of primary importance in resolving RS virus infection of BALB/c mice. Immunocompromised as well as immunodeficient BALB/c mice have each been shown to be unable to resolve experimental RS virus infection (Taylor et al., 1985; Cannon et al., 1987). Adoptive transfer of secondary virus-specific CTLs to these animals has also been shown to clear the virus rapidly from the lungs of these otherwise persistently infected animals (Cannon et al., 1987).
The present report has presented a broad analysis of a number of responses elicited in the lungs of BALB/c mice, following experimental RS virus infection. Research is currently under way in this laboratory to assess further the importance of the various aspects of the host's immune response described in this report, both in terms of protection and recovery as well as in determining immunopathological lung injury.

We would like to express our thanks to Miss W. Brown for her assistance in preparing this manuscript. The work was supported by a Lucock Research Studentship and the Wellcome Research Foundation.

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(Received 7 July 1989; Accepted 28 February 1990)