Antibody Production in Mice Chronically Exposed to Fresh Cigarette Smoke

Cigarette smoking has been found to be associated with an increased incidence of respiratory infection[1-3], and recent evidence suggests that pulmonary defence mechanisms may be impaired in cigarette smokers[4]. Several effects of cigarette smoke on alveolar macrophages and the bacteriocidal activity of the lungs have been examined[5-7]. However, considerably less is known of the effects of cigarette smoking on adaptive immune responses.

FINKLEA et al.[8] have reported that human cigarette smokers have a decreased serum antibody response to influenza virus infection or vaccination. Studies in this laboratory on the distribution of antibody-forming cells in mice chronically exposed to cigarette smoke have also indicated that the humoral immunity is impaired[9,10]. It has also been found that cigarette-smoke exposure decreases the antibody response of mice immunized i.p. with sheep erythrocytes[11]. In this study we report the serum antibody response of mice chronically exposed to fresh cigarette smoke, after the introduction of immunogen into the respiratory system. The direct effect of cigarette smoke on antibody and the antibody-forming cells has also been studied.

Materials and methods. C 57 Black mice were exposed to fresh cigarette smoke as previously described[9]. The stock cigarette smoke solution used was prepared as reported previously[12]. Mice were immunized intratracheally with 10^8 SRBC, and bled 1 week later at the peak of the primary response[13].

Haemagglutinating antibody was measured by serially diluting 0.02 ml of serum in an equal volume of PBS containing 1% normal rabbit serum. For the reaction 0.02 ml of 0.25% SRBC was added to the dilutions. Haemolytic antibody was measured in the same manner using PBS as a diluent and adding 0.02 ml of guinea-pig serum for complement and 0.02 ml of 1% SRBC. The highest dilutions showing complete lysis and definite agglutination were read after 1 h at 37 °C.

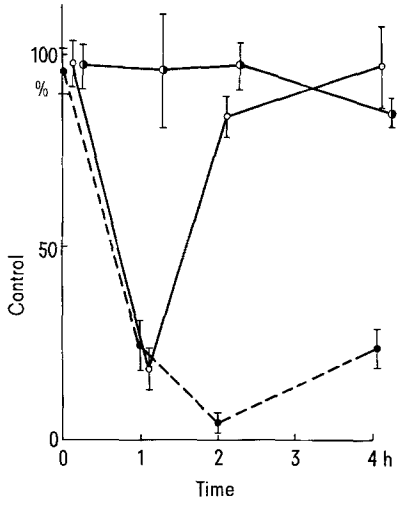
PFC preparations were made from the spleens of immunized mice and the PFC were measured as described elsewhere[13]. Rabbit anti-SRBC serum was fractionated by Sephadex G 200 chromatography, and fractions containing 19 S and 7 S antibody were collected and concentrated by ultrafiltration[14].

Results. Mice were exposed to cigarette smoke for 19 weeks before receiving an intratracheal inoculation of 10^8 SRBC. 1 week later the serum antibody activities were measured (Table). The cigarette smoke-exposed mice had lower haemagglutinating and haemolytic antibody titres than age-matched mice.

The effect of cigarette smoke solution on antibody activity was examined using the rabbit anti-SRBC antiserum fractions. Haemagglutinating and haemolytic antibody could be detected in both the 7 S and 19 S fractions. The fractions were incubated for 1 h at 37 °C in a final concentration of 1:2.5 of a fresh stock smoke solution. More fresh smoke solution was then added to a final concentration of 1:2.5 of the fresh smoke solution, and the fractions incubated for another hour. The haemagglutinating and haemolytic antibody activities were measured after the 1st and 2nd h and compared to appropriate controls. No loss of antibody activity was found due to the smoke exposure in vitro.

Antibody-forming cell preparations were suspended in final dilutions of 1:2.5, 1:10 and 1:100 of fresh smoke solution, and incubated for 0, 1, 2 or 4 h before assaying for PFC. Except for the assay performed immediately after the addition of smoke solution, the cells were resuspended in fresh medium before the PFC assay. The cigarette smoke solution had no immediate effect on the number of PFC (Figure), but after continued incubation the number of PFC showed a transient depression. The extent of the inhibition and the rate of recovery was dependent on the concentration of the smoke solution (Figure). A further decrease rather than recovery of the numbers of PFC was found if the cells that had been incubated in the 1:10 smoke solution for 1 h were retreated with a fresh 1:10 smoke solution for another hour.

Discussion. The serum antibody response to SRBC introduced to the respiratory tract was decreased in mice chronically exposed to cigarette smoke. We have previ-



Effect of cigarette smoke solutions on plaque-forming cells in vitro. Each result is the mean ± S.E. of the values from 6 cultures. 3 dilutions of smoke solution were used: ●, 1:2.5; ○, 1:10; ◑, 1:100.

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ously reported that organ PFC responses in mice chronically exposed to cigarette smoke were impaired[9,10] and the depression in the serum antibody levels shown here indicates that the decrease in the numbers of antibody-forming cells in the organs is not compensated for by an increase in antibody produced per cell, or by antibody-producing cells in an organ not examined.

The direct effect of cigarette smoking on antibody levels appears negligible, as judged by the failure of vigorous treatments with smoke solutions to inactivate either the haemagglutinating or haemolytic antibody. The antibody-producing cells exhibited a degree of sensitivity to high concentrations of cigarette smoke solutions, and the direct effect of cigarette smoke on plasma cells in the respiratory system must be considered as a possible factor moderating immune function in the lungs of cigarette smokers. However the direct effect of cigarette smoke on antibody-forming cells elsewhere in the body is probably negligible.

Antibody response of mice chronically exposed to cigarette smoke[a]

	Smoke exposed	Control
Haemolysin	0.22 ± 0.14	1.25 ± 0.45
Haemagglutinin	1.45 ± 0.24	3.40 ± 0.67

[a] Mice were exposed to cigarette smoke for 19 weeks and inoculated intratracheally with SRBC. Each result is the mean ± S.E. of the $\log_2$ serum titres of groups of 9–10 mice, measured 1 week after the inoculation. The differences between the titres of the smoke-exposed and control mice were significant as judged by Student's *t*-test ($p < 0.05$).

Zusammenfassung. Die Bildung von Antikörpern im Serum, hervorgerufen durch Inokulation von Schaferythrocyten in der Mäusetrachea, wurde durch chronische Inhalation von Zigarettenrauch vermindert.

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[16] Acknowledgments. The work was supported by a grant from the Australian Tobacco Research Foundation. WRT is in receipt of a C.S.I.R.O. Post-graduate studentship. The authors wish to thank Miss LYN ROBERTS for her skilled technical assistance.

Antiserum Inactivation of Hematopoietic Colony Forming Stem Cells from Mice with Rauscher Leukemia

Previous reports from our laboratory and others have indicated that the pluripotent hematopoietic colony forming stem cell (CFU-S) in the mouse spleen is a possible target cell for Rauscher leukemia virus[1–3]. Injection of the Rauscher virus (RLV) has been shown to effect this cell compartment in several ways, including inducing an oscillatory change in the proportion of spleen cells which are in the CFU-S compartment[4] and an eventual overall increase in the absolute number of CFU-S concomitant with the splenomegaly aspect of the disease[3–6]. Other studies concerned with the transplantation of CFU-S from Rauscher leukemic mice have shown the transplantation efficiency (f-factor) to be lowered by the leukemia, suggesting that at least a portion of that compartment was directly affected by the virus and may consist of transformed cells[1]. In order to obtain further evidence for the possible existence of transformed cells among the CFU-S of the Rauscher leukemic mouse, we have carried out additional experiments to determine if a

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Effect of antiserum incubation on the colony forming stem cells of the Rauscher leukemic mouse spleen

Incubation medium	No. of colonies formed per 10^6 transplanted cells at 8 days after transplantation		
	8-Day leukemic mouse donor	21-Day leukemic mouse donor	Normal mouse donor
Hanks' solution	37.5 ± 3.9[a]	13.4 ± 1.3	120.8 ± 5.9
Normal serum	38.6 ± 3.1	13.1 ± 1.3	120.8 ± 9.8
Antiserum	26.0 ± 2.9	5.7 ± 1.0	135.9 ± 7.1
Inhibition of colony formation by antiserum (%)	32	56	none

[a] Mean ± 1 standard error of 17 or more separate determinations.