Adjuvant Activity of Ricin as Measured by Jerne's Plaque Technique

Many substances have been reported to act as immunological adjuvants. We report that a toxic protein, ricin from *Ricinus communis* L., exerts an adjuvant activity expressed in terms of an increase in the number of plaque-forming cells as measured by Jerne's plaque technique. A ricin preparation was kindly supplied by Prof. M. FUNATSU and Dr. G. FUNATSU, Faculty of Agriculture, Kyushu University. This is a highly purified and toxic protein (MLD_{50} 0.2 μg per mouse) of a molecular weight 60,000 and an isoelectric point 5.9, which possesses no hemagglutinating and proteolytic activities[1,2].

CFI mice weighing about 30 g were injected either i.p. with 0.2 ml or i.v. with 0.05 ml of a saline containing various amounts of ricin and at the same time i.v. with 0.1 ml of a 20% sheep red blood cell suspension (4×10^8 cells). 4 days after the injections antibody-forming cells of the spleen were estimated according to the JERNE's plaque technique[3].

The group which had received 0.02 μg ricin along with the sheep red blood cells showed a significant increase in the number of plaque-forming cells as compared with a control group ($P < 0.001$), indicating that ricin possesses an adjuvant activity (Figure 1). Results shown in the Table indicate that a very small amount of ricin (0.02 μg) exerts a comparable adjuvant effect with that of 200 μg of wax D.

Examinations of the data in the Table in more detail reveals that the number of whole viable spleen cells was increased significantly when mice were injected with the red blood cells plus wax D ($P < 0.01$), while no significant increase was observed with the group receiving the red blood cells plus ricin ($P > 0.2$). The data indicate also that a significantly larger number of plaques per 10^6 spleen cells was observed in the group receiving ricin than that receiving wax D ($P < 0.02$), while, when compared on the basis of the whole spleen, no significant

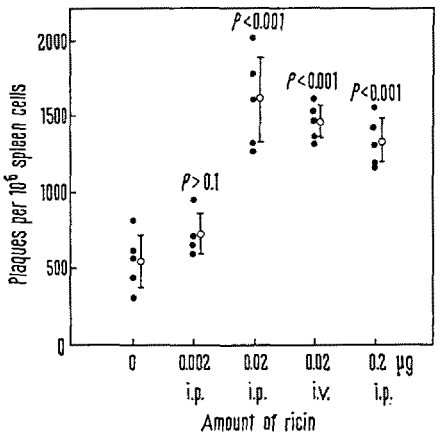

Fig. 1. Numbers of plaque-forming cells of the spleen of mice injected either i.p. or i.v. with a saline containing various amounts of ricin. At the same time mice were injected i.v. with sheep red blood cells (4×10^8).

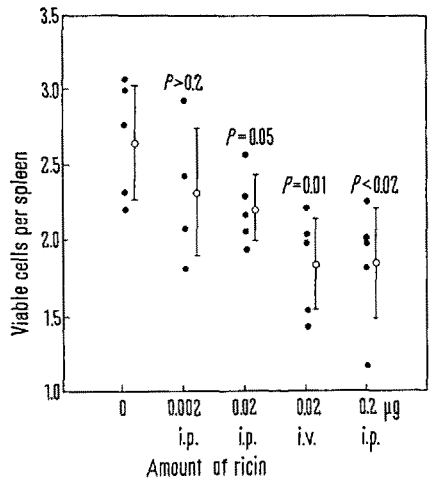

Fig. 2. Numbers of viable spleen cells of mice counted 4 days after i.p. or i.v. injections of various amounts of ricin. At the same time mice were injected i.v. with sheep red blood cells (4×10^8).

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Mean numbers (± S.D.) of viable spleen cells and plaque-forming cells after the injection of sheep red blood cells (sRBC) with adjuvants

Group	No. of viable cells per spleen ($\times 10^8$)	No. of plaques per 10^6 viable cells	per spleen
Saline	0.99 ± 0.23	3.2 ± 0.9	300 ± 77
sRBC	1.94 ± 0.26	698.7 ± 72.1	135,375 ± 23,985
Wax D	0.95 ± 0.13	1.7 ± 0.5	156 ± 41
sRBC + wax D	2.78 ± 0.09 (< 0.01)[a]	1,017.6 ± 146.3 ($= 0.02$)[a]	283,783 ± 48,035 (< 0.01)[a]
Ricin	0.83 ± 0.21	1.5 ± 0.4	110 ± 12
sRBC + ricin	1.72 ± 0.06 (> 0.2)[a]	1,503.6 ± 135.0 (< 0.001)[a] (< 0.02)[b]	257,975 ± 29,743 (< 0.01)[a] (> 0.5)[b]

4 mice were used for each group. 200 μg of wax D suspension or 0.02 μg of ricin were injected i.p. [a] P values (Student's t-test)-sRBC + wax D or ricin vs. sRBC. [b] P values – sRBC + ricin vs. sRBC + wax D.

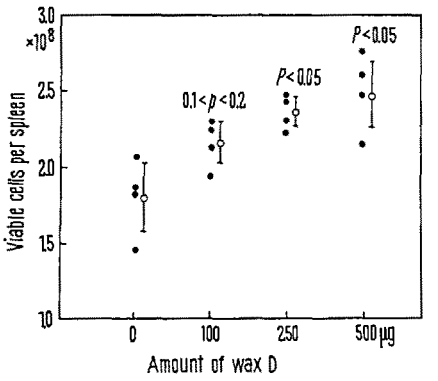

Fig. 3. Numbers of viable spleen cells of mice counted 4 days after the i.p. injection of various amounts of wax D and i.v. injection of sheep red blood cells (4×10^8).

difference in the number of plaques was observed between the two groups ($P > 0.5$).

These data suggest that ricin might exert a cytotoxic effect, which might be the basis for its adjuvant effect. Preliminary data supporting this possibility were obtained as follows. Mice were injected with the sheep red blood cells plus various amounts of either ricin or wax D. As shown in Figure 2, the numbers of whole viable cells were significantly decreased with the increase in the amount of ricin injected. In contrast, as shown in Figure 3, the number of whole viable spleen cells was significantly increased with the increase in the amount of wax D[4].

Zusammenfassung. Es wird gezeigt, dass Injektion von Rizinus-Extrakt die Plaque-Bildung im Jerne-Test fördert, was dafür spricht, dass die zytostatische Wirkung des Rizinus mit der Adjuvansfähigkeit gekoppelt ist.

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Research Institute for Diseases of the Chest, Faculty of Medicine, Kyushu University, Fukuoka (Japan), 13 August 1970.

[4] We are greatly indebted to Drs. M. FUNATSU and G. FUNATSU for their supply for ricin and to Miss Y. NAGAKAWA for her excellent technical assistance.

Placental Transfer of a Human IgG2 Monoclonal Protein

Among the four known human IgG heavy-chain subclasses[1,2] only IgG1 and IgG3 are known to be transferred across the placenta[3].

The finding during routine testing of a monoclonal IgG2 L protein in the serum of a healthy woman in her 6th month of pregnancy[4] provided a unique opportunity for the study of the placental transfer of this subclass.

Material and methods. Serum samples. Maternal serum samples were collected at the day of birth, and 3 months, 2, and 3 years after. All immunoglobulins remained quantitatively stable during the period of observation.

Serum samples were also collected from the infant, at the day of birth (cord's blood), and afterwards, at days 15, 30, 60, 90, 120, 150 and 215.

Electrophoretic and immunoelectrophoretic studies. All serum samples were studied electrophoretically on cellulose acetate[5,6] and immunoelectrophoretically in agar, according to the microtechnique described by SCHEIDEGGER[7], using horse anti-whole human serum.

Quantitative assay of immunoglobulins. Immunoglobulins were quantitated by radial immunodiffusion[8].

Heavy and light chain typing of the monoclonal protein. The preparation of specific anti-heavy chain subclass sera has been described in detail elsewhere[9]. Anti-whole light chain antiserum, prepared in a rabbit by injection of isolated light chains obtained after reduction and alkylation of normal IgG[10], was rendered specific for κ- and λ-chains by absorption with isolated IgG monoclonal proteins of the opposite light chain type. Due to the presence of normal IgG, classification of the monoclonal protein was only possible by immunoelectrophoresis.

Quantitative assay of IgG subclasses. Titration of IgG subclasses was done as described elsewhere[9]. Titres were transformed into percentages of a normal standard after correction for total IgG content.

Calculation of half-lives for circulating IgG and IgG2 in the infant. The period of time necessary for a 50% decrease in circulating IgG and IgG2 was determined from plots of concentration versus time, where the slopes were drawn so as to represent an average rate of decline at total IgG levels higher than 500 mg/100 ml.

Results. A monoclonal component migrating in the fast γ-region was detected both in mother and infant's sera by cellulose acetate electrophoresis and immunoelectrophoresis. The abnormal protein could still be detected in infant's samples collected at days 120 and 150 after birth (by immunoelectrophoresis only, in the last). This protein was typed in both mother and infant's serum samples as IgG2 L. The results of immunoglobulin assay in serum samples obtained from mother and infant are shown in Table I.

IgG subclasses were titrated in serum samples collected from the mother at day of birth and from the infant at days 30, 60, 120 and 215 after birth, and results are given in Table II.

The results of the quantitative assay of IgG, IgG2, and IgM in infants samples are plotted versus time in the Figure. Half lives of 45 and 60 days were calculated for IgG and IgG2, respectively.

Discussion. The selectivity of the placental transfer of IgG[11] was observed in the present case, as shown by the similarity of the levels of this immunoglobulin in mother's and infant's sera at day of birth, and by the very low levels of IgA and IgM detected in the infant. IgG levels decreased during the first 3 months of life, as expected[12], and levels of IgA increased only slightly up to day 215, also in agreement with reported data for that immunoglobulin[12]. Less expected was the very early rise of IgM, reaching normal adult levels as soon as day 30. This data was confirmed immunoelectrophoretically.

Information about the placental transfer of IgG sub classes was obtained in 2 different ways. The detection

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