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SHORT COMMUNICATIONS

Modulation of Delayed Hypersensitivity in Mice by Cobra Venom Factor

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The effect of administration of purified cobra venom factor (CoF) on both induction and expression of delayed hypersensitivity (DH) to sheep red blood cells in mice was studied. Injection of CoF before immunization resulted in enhanced DH, whereas CoF treatment before elicitation suppressed the response. These effects could not directly be associated with reduced serum C_3 levels. CoF induced a stimulation of the mononuclear phagocytic system as measured by the clearance of colloidal carbon from the blood. A relation between this stimulatory effect and the modulation of DH is discussed. It is suggested that the macrophage is a major target cell of this CoF action.

INTRODUCTION

There is evidence from *in vivo* experiments that complement (by its factor C_3) is involved in the induction of thymus-dependent antibody formation in mice (1). The role of C_3 in cell-mediated immune reactions is still controversial. Decomplementation of rats by injections of immune complexes or aggregated gammaglobulin before challenge with antigen inhibited delayed hypersensitivity (DH) (2). In guinea pigs specific C_3 depletion by injecting anti-C_3 serum before challenge for DH or a nonspecific inflammatory stimulus suppressed the skin reactions (3). In the same animal species treatment with cobra venom factor (CoF) delayed the rejection of an allograft (4). On the other hand immuno-histological studies of DH lesions (5) and CoF treatment of guinea pigs before elicitation of DH (6, 7) failed to demonstrate an obligatory role for C_3 in the expression of DH. Studies on the induction of other T-cell-dependent phenomena in mice revealed that C_3-depletion by CoF decreased antibody formation particularly the secondary response (1), whereas allograft rejection and host-versus-graft reactivity were unaffected (8). This might imply that the function of Lyt-1^+ cells which exert helper and DH reactivity involves serum C_3, whereas Lyt-$2,3^+$ (effector) cells function also in the absence of C_3 (9, 10). However T-cell education to sheep red blood cells (SRBC) in irradiated animals, which also concerns helper T cells, was unimpaired by treatment of mice with CoF (8). In the present study the effect of C_3 depletion on both induction and expression of DH in mice is investigated.

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MATERIALS AND METHODS

Mice. Male F_1 (BALB/c × Swiss) mice were bred and maintained at the Laboratory of Microbiology, Utrecht, The Netherlands and used at an age of 14 weeks.

CoF. CoF (Naja naja, Sigma, St. Louis, Mo.) was purified as described by Ballow and Cochrane (11) on DEAE-cellulose followed by G_{200} Sephadex gel filtration (Sephadex, Pharmacia, Sweden). Mice received one or two intraperitoneal injections of 2 units CoF in 0.5 ml of saline.

Assay for DH. Mice were immunized intracutaneously on the abdomen with 2×10^7 sheep red blood cells (SRBC) in saline divided over four sites. Five days later the animals were challenged for DH by subcutaneous injection of 1×10^8 SRBC in a total volume of 0.05 ml in the left hind footpad. The footpad swelling was measured after 24 hr with a semielectronic paw meter (12).

Determination of hemolytic C_3. Mice were bled by retroorbital puncture. Hemolytic C_3 levels of the sera obtained were determined with pooled human R_3 serum as source of other complement components (13).

Assay of activity of the mononuclear phagocytic system. The activity of the mononuclear phagocytic system (MPS, 14) was determined by the clearance of intravenously injected colloidal carbon (Pelikan ink, C11/1431a, Gunther Wagner, Hannover, Germany, 160 mg/kg body wt) and expressed as phagocytic index

$$K = \frac{\log C_0 - \log C_t}{t},$$

where C_0 and C_t are the carbon concentrations in the blood at zero time and time t (15).

RESULTS AND DISCUSSION

Two intraperitoneal (ip) injections of 2 units of CoF each before intracutaneous (ic) immunization with 2×10^7 SRBC caused a slight albeit significant enhancement of DH (Fig. 1). The serum C_3 levels of CoF-treated mice at the time of immunization and measuring DH mounted from <1 to 19 ± 1% of the saline-treated controls. Administration of 2 units of CoF simultaneously with challenge for DH resulted in nondemonstrable C_3 levels during the development of DH and a significant suppression of this reaction. These results indicate that there is no correlation between the severity of a DH reaction and the subsisting C_3 level and suggest another mechanism underlying the stimulation and suppression of DH, depending on the time of CoF administration. Other agents sharing with CoF the properties of immunomodulation and alternate complement pathway activation, as BCG (16–18), carrageenan (18–20), *Corynebacterium parvum* (16, 18), lipopolysaccharide (18, 21), and dextran sulfate (16, 18, 22) are known to be stimulators of the MPS and inducers of lysosomal enzyme release (18). This suggests that macrophage activation and not a direct influence on T-cell functions might be the underlying cause of the immunomodulating properties of CoF. To investigate this further CoF was injected in nonimmunized mice following the same schedule as used in the DH experiments. At the critical times the MPS activity was estimated by determining the clearance of colloidal carbon (15). Table 1 shows that the phagocytic index is significantly increased 2 hr after the last of two CoF injections and that it reaches about normal values after 5 to 6

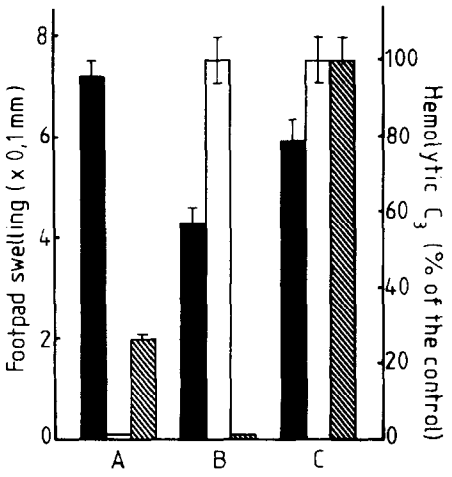

FIG. 1. Effect of CoF treatment on delayed hypersensitivity and hemolytic C_3. Groups of 28 mice were immunized for DH (■) and received intraperitoneal injections of 2 units of CoF 24 and 2 hr before immunization (A), a single ip injection of 2 units of CoF simultaneously with challenge for DH (B), or saline (C). Groups of six mice were treated with CoF and saline following the same schedules and hemolytic C_3 was determined at the time of immunization (□) or measurement of DH (▨). Bars indicate the standard error of the mean. The two-tailed Student's t test was performed to determine the P values for differences in footpad swelling (A, $P < 0.05$; B, $P < 0.02$).

days. This means that pretreatment with CoF in the DH experiment greatly enhances MPS activity at the time of immunization. The results of Table 1 indicate further that a single injection of CoF simultaneously with challenge for DH results in mounting phagocytic indices during the development of DH. The stimulation of the MPS might be mediated by complement split products as C_{3b}, which was shown to induce lysosomal enzyme release from murine macrophages *in vitro* (23, 24). These lysosomal enzymes (24, 25) and CoF (26) can subsequently

TABLE 1

Carbon Clearance of CoF and Saline-Treated Mice at Times Corresponding to Immunization, Challenge for DH, and Measurement of Footpad Swelling[a]

Treatment (ip) at (hr)		Phagocytic index K at time of immunization 0 hr	Treatment (ip) at time of challenge 120 hr	Phagocytic index K at 122 hr	Phagocytic index K at time of measurement of DH 144 hr
−24	−2				
CoF	CoF	0.0632 ± 0.0015[b,*]	Saline	0.0347 ± 0.0018	0.0473 ± 0.0038**
Saline	Saline	0.0375 ± 0.0035	CoF	0.0471 ± 0.0018*	0.0669 ± 0.0019*
Saline	Saline	0.0375 ± 0.0035	Saline	0.0350 ± 0.0019	0.0349 ± 0.0029

[a] Groups of six mice received two intraperitoneal injections with 2 units of CoF or saline. Two hours later the clearance of intravenously injected colloidal carbon of some groups was determined. The remaining groups received another i.p. injection of 2 units of CoF or saline at 120 hr and the phagocytic index was determined at 122 or 144 hr.

[b] Mean ± standard error of the mean, P values were obtained by performing two-tailed Student's t test.

* $P < 0.0001$.

** $P < 0.03$.

generate chemotactic factors. This might imply that macrophages will be trapped at the injection site of CoF resulting in a decreased availability of macrophages at the immunization site, favoring the induction of DH (27, 28). A reduced availability of macrophages at the site of challenge injection can explain the impaired expression of the response as consequence of late CoF injection.

Our results strongly suggest that CoF by depleting C_3 modulates the induction and expression of DH. They are, however, not in accordance with the suggestion by Rumjanek *et al.* (8), that effects on T-cell functions described for CoF are due to impurities in the CoF preparation used. It is rather likely that the effects of CoF on the triggering of T cells involved in DH and on the expression of DH, are mediated by macrophages. Very recently a similar explanation was given for the effect of CoF on early IgM and IgG responses in mice, although not confirmed experimentally. A definitive conclusion concerning a possible direct involvement of C_3 in T-cell functions, however, will have to be deferred, as it was shown that macrophages are able to produce C_3 (30–32). The influence of *in vivo* CoF treatment on C_3 production by macrophages will have to be elucidated first.

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