Clin. exp. Immunol. (1972) **11**, 579–584.

DEPRESSION OF DELAYED HYPERSENSITIVITY BY PRETREATMENT WITH FREUND-TYPE ADJUVANTS

III. DEPRESSED ARRIVAL OF LYMPHOID CELLS AT RECENTLY IMMUNIZED LYMPH NODES IN MICE PRETREATED WITH ADJUVANTS

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(*Received* 17 *February* 1972)

SUMMARY

Pretreatment of mice with Freund's complete adjuvant (FCA) or *Corynebacterium parvum* depresses the contact sensitivity which otherwise follows immunization with picryl chloride in FCA (PCL/FCA). In contrast, *Bordetella pertussis* is inactive. The hypothesis that this depression was partly due to the failure of lymph nodes immunized with PCL/FCA to collect lymphoid cells was tested. Mice were pretreated with FCA, *C. parvum* or *B. pertussis*, subsequently immunized with PCL/FCA, and injected with ^{51}Cr-labelled normal lymph node cells.

Pretreatment with FCA 10 days before immunization depressed the arrival of labelled cells at draining nodes immunized with PCL/FCA by 10–19%.

Pretreatment intravenously with *C. parvum* 4 days before immunization virtually abolished the increased arrival at draining lymph nodes which is otherwise seen 2 and 4 days after immunization, but had little effect on the increased arrival seen 1 day after immunization. In contrast, *B. pertussis* caused a slight but significant depression at day 1, but had no effect 2 and 4 days after immunization.

The depression of contact sensitivity by these three bacterial adjuvants was paralleled by their depression of the arrival of labelled lymph node cells at recently immunized nodes, and supported the hypothesis that failure of recently immunized lymph nodes to collect lymphoid cells is part of the mechanism of depression of delayed hypersensitivity by bacterial adjuvants, and in particular, by *C. parvum*.

INTRODUCTION

Pretreatment of animals with Freund's complete adjuvant (FCA) or *C. parvum* before immunization with a test antigen leads to a depression of delayed hypersensitivity skin

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reactions (Jankovic, 1962; Asherson & Allwood, 1971). In the guinea-pig, the depression caused by pretreatment with FCA is due in part to an anti-inflammatory effect of the adjuvant, and in part to a depression of the central state of immunity (Allwood & Asherson, 1971).

The cells which initially divide in the induction of the delayed hypersensitivity reaction are thymus-derived cells (Davies *et al.*, 1966), and these cells occur in large numbers in the peripheral blood and the thoracic duct lymph. A failure of these cells to reach peripheral lymph nodes would provide a possible basis for the depression of the central state of immunity. This paper considers the mechanism whereby Freund's complete adjuvant depresses the central state of immunity. It examines the hypothesis that this is due to a competition between lymph nodes pretreated with FCA and the other lymph nodes for thymus-derived cells; and shows that pretreatment with FCA or *C. parvum* depresses the arrival of injected lymph node cells at lymph nodes later immunized with picryl chloride in FCA.

MATERIALS AND METHODS

Randomized CBA mice were pretreated in two ipsilateral footpads with a total of 0·04 ml FCA containing 5 mg/ml heat-killed tubercle bacilli. Ten days later, they were immunized with a total of 50 μg picryl chloride in 0·04 ml FCA into the two contralateral footpads.

In the experiments described in Tables 2 and 3, mice were pretreated with 0·5 mg heat-killed *C. parvum* (a kind gift from Dr G. Biozzi), or with $1{\cdot}2 \times 10^{10}$ *B. pertussis* (Burroughs Wellcome: Per/Vac), intravenously, and immunized 4 days later in the hind footpads with PCL/FCA as above. (See Asherson & Allwood, 1971). The treatments were given at various times so that the injection of the labelled cells could be performed on 1 day.

Cell suspensions were made from limb girdle lymph nodes from about fifteen normal mice and labelled with ^{51}Cr (Bainbridge, Brent & Gowland, 1966). 5×10^6 cells (70–80% viable by eosin dye exclusion) were injected intravenously into recipients whose lymph nodes were removed 20 hr later. See Asherson & Allwood (1972) for further details.

RESULTS

The arrival of normal lymph node cells at nodes immunized with picryl chloride in FCA: the effect of pretreatment with FCA

Lymphoid cell arrival was studied by labelling dissociated lymph node cells *in vitro* with ^{51}Cr and injecting them into recipients. The net arrival of ^{51}Cr at various organs, expressed as a percentage of the injected radioactivity, was used as a measure of the net cell arrival, and in the text, the phrase 'arrival of cells' is used as a shorthand for 'net percentage arrival of ^{51}Cr'.

Groups of six mice were pretreated with FCA in the ipsilateral footpads (control mice were left untreated), 10 days before immunization in the contralateral footpads with PCL/FCA. Labelled normal lymph node cells were injected intravenously 0, 2, 4, 7 and 11 days later. After a further 20 hr the contralateral nodes (immunized with PCL/FCA) were removed and counted.

Table 1 shows that the arrival of labelled cells at unimmunized nodes in normal unimmunized animals was 3·3%, and this arrival was unaffected by pretreatment with FCA. Immunization of normal mice with PCL/FCA increased the arrival to 9·4% at day 2, and the

TABLE 1. Arrival of normal lymph node cells at unimmunized and recently immunized lymph nodes: the effect of pretreatment with Freund's complete adjuvant

Days after immunization with picryl chloride in FCA	Arrival of lymph node cells at draining lymph nodes in mice pretreated with:		Percentage reduction
	Nil (control)	FCA	(%)
Control group (unimmunized)	3·3±0·3	3·1±0·4	4
2 Days after PCL in FCA	9·4±0·8	7·7±0·5	19++
4 Days after PCL in FCA	10·2±1·1	9·2±1·3	10
7 Days after PCL in FCA	12·2±1·4	10·5±1·3	14+
11 Days after PCL in FCA	12·8±2·1	10·9±1·1	14+

Mice were pretreated with Freund's complete adjuvant into two ipsilateral footpads, or left untreated, 10 days before immunization in the two contralateral footpads with picryl chloride in Freund's complete adjuvant. The injections were arranged so that all the mice received labelled normal lymph node cells on the same day. The figures show the mean net percentage arrival (± S.D.) of ^{51}Cr labelled lymph node cells at the contralateral (limb girdle + popliteal) lymph nodes draining the footpads injected with PCL in FCA, and taken at the stated time following injection.

+ $0{\cdot}01 < P < 0{\cdot}05$.

++ $0{\cdot}001 < P < 0{\cdot}01$.

P calculated by Student's *t*-test.

arrival remained high for at least 12 days. Dresser, Taub & Krantz (1970) made similar observations. Pretreatment with FCA depressed the arrival at nodes immunized with PCL/FCA by 10–19% from day 2 onwards.

This experiment was repeated using time intervals of 0, 1, 2, 3 and 10 days between the immunization with PCL/FCA and the injection of labelled cells. There was no significant depression of arrival on days 0, 1, or 10 and significant depression of 11% and 19% on days 2 and 3. It was concluded that pretreatment with FCA before immunization with PCL/FCA depressed the arrival of lymph nodes at immunized nodes.

The arrival of normal lymph node cells at nodes immunized with picryl chloride in FCA: the effect of pretreatment with C. parvum or B. pertussis

Groups of six mice were pretreated with *C. parvum* or *B. pertussis* intravenously, while control mice were injected with saline. Four days later, the mice were immunized with PCL/FCA. After a further 1, 2 or 4 days, labelled normal lymph node cells were injected intravenously, and the draining (popliteal) lymph nodes, and the distant (limb girdle) lymph nodes were removed.

Table 2 shows that 4 days after immunization with PCL/FCA, the arrival of lymph node cells at the draining lymph nodes increased by about three-fold. Pretreatment with *C. parvum* (but not with *B. pertusis*) virtually abolished the increased arrival which normally follows 2 and 4 days after immunization with PCL/FCA. Both agents had little effect on the increase in cell arrival 1 day after immunization with PCL/FCA. *B. pertussis* actually caused a slight but significant depression. It was concluded that pretreatment with *C. parvum* intravenously depressed the arrival of lymph node cells at nodes draining the site of injection of PCL/FCA. The right-hand part of Table 2 shows the arrival of cells at the distant

TABLE 2. Arrival of normal lymph node cells at unimmunized and recently immunized lymph nodes: the effect of pretreatment with *C. parvum* and *B. pertussis*

Days after immunization with picryl chloride in FCA	Arrival of lymph node cells at					
	+ 'Draining' lymph nodes in mice pretreated with			++ 'Distant' lymph nodes in mice pretreated with		
	Saline	*C. parvum*	*B. pertussis*	Saline	*C. parvum*	*B. pertussis*
Control group (unimmunized)	1·1±0·2	0·8±0·4	1·0±0·1	6·8±0·7	3·6±0·2	5·7±0·3
1 Day after PCL in FCA	2·4±0·2	2·2±0·4	2·0±0·4	5·9±1·1	4·6±0·5	6·0±1·0
2 Days after PCL in FCA	2·7±0·3	1·3±0·9	2·4±0·4	6·4±1·4	3·8±0·5	6·2±0·9
4 Days after PCL in FCA	3·4±0·4	1·1±0·1	3·2±0·5	6·8±1·0	2·7±0·3	6·1±0·6

Mice were pretreated intravenously with *C. parvum*, *B. pertussis*, or saline, 4 days before immunization in the two hind footpads with picryl chloride in Freund's complete adjuvant. The +draining popliteal lymph nodes, and the ++distant limb girdle lymph nodes were taken the stated time after injection of PCL/FCA. Note that the draining nodes are different from those in experiment 1. Table 3 shows the arrival of lymph node cells at liver and spleen in the same animals.

nodes was virtually unaffected by immunization with PCL/FCA. *B. pertussis* did not affect this arrival. However, *C. parvum* depressed the arrival of lymph node cells at distant lymph nodes by about 50%.

The spleens of the mice pretreated with *C. parvum* and *B. pertussis* were greatly enlarged. Table 3 shows, however, that there was no consistent effect of pretreatment upon the arrival of lymph node cells at the spleen. It also shows that pretreatment with these bacterial adjuvants did not increase liver arrival.

TABLE 3. Arrival of normal lymph node cells at liver and spleen: the effect of pretreatment with *C. parvum* and *B. pertussis*

Days after immunization with picryl chloride in FCA	Arrival of lymph node cells at liver and spleen in mice pretreated with					
	Liver			Spleen		
	Saline	*C. parvum*	*B. pertussis*	Saline	*C. parvum*	*B. pertussis*
Control group (unimmunized)	21·1±1·4	23·2±1·1	23·5±2·1	20·5±1·8	21·2±1·8	20·0±0·8
1 Day after PCL in FCA	24·2±1·4	25·8±0·1	25·7±0·7	20·9±1·7	19·3±0·9	19·7±0·8
2 Days after PCL in FCA	24·6±1·5	25·1±1·8	25·7±0·7	19·8±1·4	19·3±0·4	18·9±1·3
4 Days after PCL in FCA	23·9±0·8	19·4±2·1	24·8±0·8	19·5±1·2	26·9±2·4	20·1±1·1

DISCUSSION

In the mouse, pretreatment with FCA causes a moderate depression of the contact sensitivity which otherwise follows immunization with picryl chloride in FCA, while pre-treatment with *C. parvum* causes greater depression. In contrast, *B. pertussis* has no effect. By analogy with the guinea-pig, part of these effects may be due to a depression of the central state of immunity (i.e. a defect in the cell population(s) which initiate the delayed hypersensitivity skin reaction) and part to an anti-inflammatory action. One possible explanation of the depressed central state of immunity is a failure of thymus-processed cells needed for the induction of delayed hypersensitivity to move to recently immunized lymph nodes in animals pretreated with adjuvant. This hypothesis was tested by injecting dissociated labelled lymph node cells into mice pretreated with adjuvants and looking at the arrival at lymph nodes subsequently immunized with PCL/FCA.

The results showed that pretreatment with FCA depressed the arrival at recently immunized lymph nodes by about 15%. In contrast, *C. parvum* depressed the arrival by 66%, and virtually abolished the ability of immunization with PCL/FCA to increase the arrival of lymph node cells. *B. pertussis* had little effect on cell arrival. These results correlate with the effect of these agents on contact sensitivity, and are compatible with the hypothesis that part of the depression of contact sensitivity caused by pretreatment with adjuvants is due to an impaired ability of recently immunized lymph nodes to collect lymphoid cells. The hypothesis is particularly germane to the depression caused by *C. parvum* as this agent has a big effect on cell arrival.

The depression of arrival caused by FCA may have a simple explanation in a competition between the lymph nodes pretreated with FCA and the more recently immunized lymph nodes, for the labelled lymph node cells. In the case of *C. parvum*, the failure of cells to arrive at recently immunized lymph nodes is not due to these cells lodging in the spleen, and the detailed mechanism remains to be elucidated.

The present hypothesis that depressed delayed hypersensitivity can be caused in part by a failure of thymus-processed cells to arrive at lymph nodes subject to antigenic stimuli, may be relevant to the explanation of some forms of antigenic competition, and depression of delayed hypersensitivity by bacteria and viruses. It may also be important in the explanation of the inhibition of graft-versus-host reactions in recipient F1 hybrid mice pretreated with *C. parvum* before injection of parental spleen cells (Howard *et al.*, 1967).

ACKNOWLEDGMENTS

We would like to thank the Medical Research Council and the Cancer Research Campaign for financial support.

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