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Characterizaton of low dose induced suppressor cells in adjuvant arthritis in rats

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SUMMARY

Characterization of suppressor cells in adjuvant arthritis was performed by using highly susceptible DA strain rats. The results showed that suppressor cells were induced after a single inoculation of subarthritogenic dose of mycobacterial adjuvant. Relatively long incubation period was required for the induction of suppressor cells. Such cells were predominated in the draining lymph node and, after fractionation, only sIg^- population was effective in conferring unresponsiveness. *In vivo* irradiation or hydrocortisone treatment suggested that low dose induced suppressor cells were resistant against such immunosuppressive treatments. In addition, by using alkyldiamine as a non-mycobacterial arthritogenic adjuvant, it was suggested that unresponsiveness induced by low dose priming with mycobacterial adjuvant was antigen specific.

Keywords adjuvant arthritis rats unresponsiveness suppressor cells

INTRODUCTION

Adjuvant arthritis can be induced by a single injection of mycobacterial adjuvant (Pearson, 1956). Although the pathogenesis of adjuvant arthritis in rats is thought to have resulted from cell-mediated immune responses (Waksman, Pearson & Sharp, 1963; Pearson & Wood, 1964), precise effector or regulator cells are not clearly identified. Arthritogenic reaction of the adjuvant can be prevented by pre-treating animals in several different ways (reviewed by Eugui & Houssay, 1975). Eugui & Houssay (1975) demonstrated that unresponsive state could be induced by subarthritogenic dose of mycobacterial adjuvant and this unresponsiveness was transferred by draining lymph node cells.

The aim of this study is to confirm the work of Eugui & Houssay (1975) and to further characterize the origin and the nature of the suppressor cells induced by subarthritogenic dose of mycobacterial adjuvant in highly susceptible DA strain rats.

MATERIALS AND METHODS

Animals. Female DA rats (originated from John Curtin School of Medical Research, Australian National University, and maintained in our laboratory by sib-mating for more than 10 years), 8 weeks old, weighing approximately 200 g were used throughout. This strain showed 100% incidence with high severity of arthritis after a single inoculation of arthritogenic dose of mycobacterial adjuvant. They were fed *ad libitum* with standard laboratory chow and water.

Adjuvant and inoculation. Adjuvant was prepared by dispersing required amount of ground heat-killed *Mycobacterium tuberculosis* human strain Aoyama B (supplied through the courtesy of

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the Chemo-Sero-Therapeutic Research Institute, Kumamoto) in mineral oil (Paraffin flussig, Art. 7161, Merk). To examine the specificity of unresponsiveness, *N,N*-dioctadecyl-*N'*,*N'*-bis(2-hydroxyl) propandiamine, which is known as non-mycobacterial arthritogenic adjuvant (Chang, Pearson & Abe, 1980) was used. Optimum dose for the induction of arthritis and that for the induction of unresponsiveness were determined by preliminary experiments. For the induction of unresponsiveness, 0·003 mg mycobacteria in 0·1 ml mineral oil was inoculated intradermally into right hind foot pad of each rat. Control rats were inoculated 0·1 ml mineral oil alone by the same manner. Reinoculation with arthritogenic dose (0·3 mg mycobacteria/0·1 ml of oil/rat, or 10 mg alkyldiamine/0·2 ml/rat) was performed by an intradermal injection at the base of the tail.

Evaluation of arthritis. All rats were examined regularly for arthritis after adjuvant injection. At each examinaton, the severity of the arthritis was evaluated by visual scoring of the joints depending on the degree of swelling and redness. Scoring ranges were as follows, wrists: 0–5, ankles: 0–10, tarsi: 0–10, each of the smaller joints (metatarsophalangeal and interphalangeal): 0–1. The lesions of injected foot and/or tail were not included in the score. Thus, the maximum possible score could be 40.

Hydrocortisone treatment in vivo. Hydrocortisone sodium succinate (Solu-Cortef, Upjohn Co., Kalamazoo, Michigan, USA) was dissolved in sterile saline at a concentration of 12·5 mg/ml. One ml of this solution was intraperitoneally injected 3 days before priming with subarthritogenic dose or 3 days before reinoculation with arthritogenic dose of the adjuvant. The dose was determined after Kayashima, Koga & Onoue (1978).

Whole body irradiation. Rats were exposed 200 or 400 rad whole body irradiation from a ^{60}Co source (Toshiba RCR-120-C1, focal skin distance; 80 cm, dose rate of 76·8 rad/min) 3 days before priming or reinoculation (Kayashima *et al.*, 1976).

Cell transfer. Popliteal lymph nodes of inoculated side, other lymph nodes (cervical and axillary nodes), or spleen were gently squashed between two frost ended slides in cold Eagle's MEM. The cell suspension was passed through four layers of surgical gauze to remove cell debris. The cells were washed three times and resuspended in the fresh medium. The cell suspension was injected intravenously via tail vein of recipient rats.

Cell fractionation. Cell fractionation into surface immunoglobulin positive (sIg^+) or negative (sIg^-) cells was performed by a rosetting procedure according to Parish & Hayward (1974a, 1974b). Briefly, SRBC coated with rabbit Ig anti-rat Ig by chromium chloride method were added to the

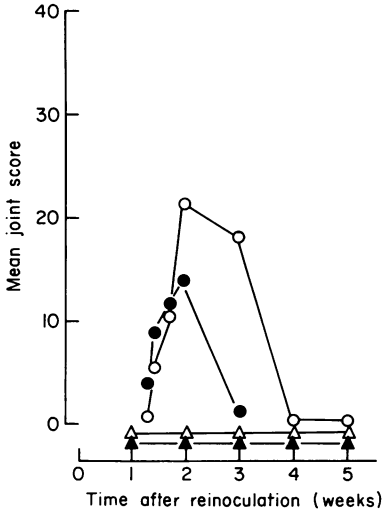

Fig. 1. Induction of unresponsiveness after priming with subarthritogenic dose of mycobacterial adjuvant. Each five rats were reinoculated with arthritogenic dose (0·3 mg/rat) of the adjuvant at 5 days (○), 2 weeks (●), 4 weeks (△) or 8 weeks (▲) after priming.

lymph node cell suspension. After incubation and centrifugation to facilitate the rosette formation, the mixture was layered onto Ficoll–Isopaque density separation medium. After centrifugation, cells in the top and the bottom layer were collected separately. Cells collected from the bottom were further treated with NH_4Cl-Tris buffered solution to remove red blood cells (Mishell & Shiigi, 1980). The cell suspensions were washed three times and adjusted to the required concentration. The efficiency of the fractionation had been tested by staining cells with horseradish peroxidase labelled rabbit Ig anti-rat Ig before and after separation and the viability of the cells was more than 95% by trypan blue dye exclusion test immediately before cell transfer.

RESULTS

Induction of unresponsiveness

Rats were primed by intradermal injection with subarthritogenic dose (0·003 mg/rat) of mycobacterial adjuvant into right hind foot pad. Rats given mineral oil alone were served as controls. Reinoculation with arthritogenic dose (0·3 mg/rat) was performed at various days after

Table 1. Adoptive transfer of unresponsiveness by draining lymph node cells obtained at 4 or 8 weeks after priming with subarthritogenic dose of mycobacterial adjuvant

Cells	No. of cells transferred	Maximum joint score*	Incidence†
—	—	39·8 ± 0·2	5/5
4 weeks	5×10^8	29·4 ± 5·6‡	5/5
8 weeks	5×10^8	0	0/5

* Mean score ± s.e. (mean).

† No. of arthritic rats/No. of recipients.

‡ $0{\cdot}05 < P < 0{\cdot}1$ when compared to the group without cell transfer (Student's t-test).

Table 2. Comparison of the efficacy of suppression by adoptive transfer of various cell sources

Cell source*	No. of cells transferred	Maximum joint score†	Incidence‡
—	—	39·8 ± 0·2	5/5
PLN	5×10^8	0	0/5
	2×10^8	0	0/5
Other LN	10×10^8	26·6 ± 3·7§	4/4
	5×10^8	30·6 ± 5·1	5/5
	1×10^8	35·4 ± 2·1	5/5
Spleen	10×10^8	35·8 + 1·3§	5/5
	5×10^8	37·4 ± 0·5	5/5

* Cells from popliteal lymph node of inoculated side (PLN), from other lymph nodes, or spleen obtained 8 weeks after priming with subarthritogenic dose of mycobacterial adjuvant.

† Mean score ± s.e. (mean).

‡ No. of arthritic rats/No. of recipients.

§ Statistically significant ($P < 0{\cdot}02$) when compared to the group without cell transfer (Student's *t*-test).

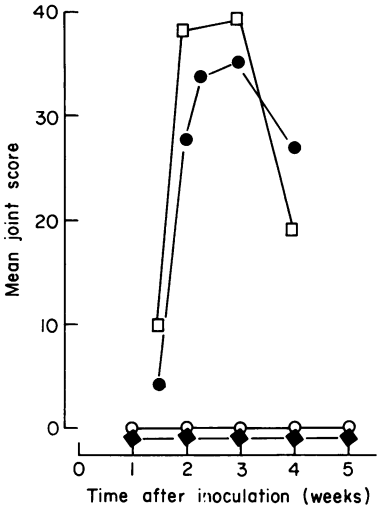

Fig. 2. Adoptive transfer of unresponsiveness by fractionated lymph node cells. Each five rats were received intravenously none (□), whole draining lymph node cells (○), fractionated suspensions of 5×10^7 sIg^- (■) or 2×10^8 sIg^+ (●) cells. Immediately after 2×10^8 cell transfer, all rats received arthritogenic dose of the adjuvant.

the priming with subarthritogenic dose. As shown in Fig. 1, the longer a time interval between priming and rechallenge was, the lower the arthritic score resulted, and complete unresponsiveness was first induced at 4 weeks.

Adoptive transfer of unresponsiveness

Draining lymph node (right popliteal node) cells were obtained from donor rats 4 or 8 weeks after priming with subarthritogenic dose of mycobacterial adjuvant. Five hundred million cells were adoptively transferred into normal syngeneic recipients and, at the same time, recipients were given intradermal injection with arthritogenic dose of mycobacterial adjuvant. As shown in Table 1,

Table 3. Effect of hydrocrotisone or irradiation on unresponsiveness

Treatment 3 days before priming	Low dose priming*	Treatment 3 days before induction	Induction	Maximum† joint score	Incidence‡
No	No	No	Yes§	39·8±0·2	5/5
No	No	Hydrocortisone	Yes	39·7±0·3	5/5
No	No	200 rad	Yes	40·0±0·0	5/5
No	No	400 rad	Yes	39·8±0·2	5/5
No	Yes	No	Yes	0	0/5
Hydrocortisone	Yes	No	Yes	0	0/5
No	Yes	Hydrocortisone	Yes	0	0/5
200 rad	Yes	No	Yes	0	0/5
No	Yes	200 rad	Yes	0	0/5
400 rad	Yes	No	Yes	0	0/5
No	Yes	400 rad	Yes	0	0/5

* Right hind foot pad injection with a subarthritogenic dose of the adjuvant.

† Mean score ± s.e. (mean).

‡ No. of arthritic rats/No. of recipients.

§ Induction of arthritis with arthritogenic dose of mycobacterial adjuvant 8 weeks after low dose priming.

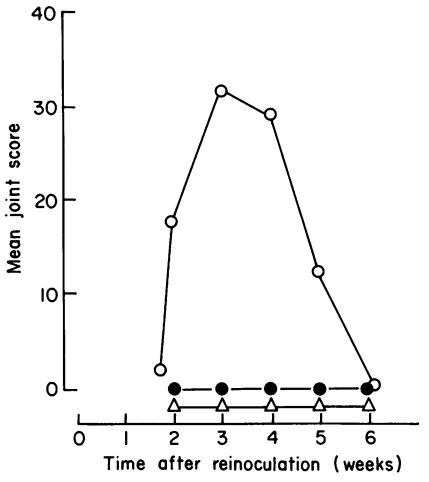

Fig. 3. Specificity of low dose induced unresponsiveness. Each five rats were reinoculated with 0·3 mg (●), 3 mg (△) of mycobacterial adjuvant or 10 mg (○) of non-mycobacterial adjuvant (*N*,*N*-dioctadecyl-*N'*,*N'*-bis(2-hydroxyl) propandiamine) 8 weeks after priming with subarthritogenic dose of mycobacterial adjuvant.

complete unresponsiveness was observed by adoptive transfer of draining lymph node cells obtained 8 weeks after low dose priming. Draining lymph node cells obtained 4 weeks after the priming were, however, not effective.

When other non-draining lymph node cells or spleen cells obtained 8 weeks after low dose priming were adoptively transferred, no or only marginal suppressive effect was observed (Table 2).

Adoptive transfer of unresponsiveness by fractionated lymph node cells

To determine which subpopulation of lymph node cells could confer unresponsiveness, draining lymph nodes cells were obtained from donors 8 weeks after priming with subarthritogenic dose of mycobacterial adjuvant and were fractionated into sIg^+ and sIg^- cell populations. Original cell suspensions or fractionated cell suspensions were injected into normal syngeneic recipient rats. Immediately after cell transfer, they received arthritogenic adjuvant. As shown in Fig. 2, unresponsiveness could be adoptively transferred solely by sIg^- cells but not by sIg^+ cells.

Effect of hydrocortisone or irradiaton on unresponsiveness

It has been reported that the suppressor T cells are more sensitive to the radiation (Tada, Taniguchi & Okumura, 1971; Kayashima *et al.*, 1976; Yamaguchi & Kishimoto, 1978) or hydrocortisone (Kayashima *et al.*, 1978) treatment than are T cells responsible for helper activities. Therefore, in the following experiments, effects of hydrocortisone or low dose irradiation on the unresponsiveness induced by subarthritogenic adjuvants were examined *in vivo*. Results were summarized in Table 3. Apparently hydrocortisone administration or irradiation 3 days before low dose priming had no effect on the induction of unresponsiveness. Same treatments done at 3 days before reinoculaton with arthritogenic dose were also not able to abrogate the unresponsive state. It is important to note that induction of arthritis by mycobacterial adjuvant *per se* was not affected by such immunosuppressive treatments, showing effector cells were intact during the experiments.

Specificity of low dose induced unresponsiveness

To examine whether unresponsiveness induced by low dose priming with mycobacterial adjuvant was antigen specific or not, rats were primed with subarthritogenic dose of mycobacterial adjuvant and, 8 weeks later, they were rechallenged with non-mycobacterial adjuvant, alkyldiamine. As shown in Fig. 3, pre-treatment with subarthritogenic dose of mycobacterial adjuvant did not affect the arthritogenicity of alkyldiamine, although it completely suppressed the response to arthritogenic dose of mycobacterial antigens.

DISCUSSION

The present study revealed that unresponsiveness to arthritogenic dose of mycobacterial adjuvant could be adoptively transferred by draining lymph node cells from unresponsive rats prepared by pre-treatment with subarthritogenic dose of the same adjuvant. Furthermore, our experiments strongly suggest that such suppressor cells are sIg^- cells, presumably T cells, of radioresistant, hydrocortisone resistant subpopulation. These results confirm and further expand the observations of Eugui & Houssay (1975) that low dose induced unresponsiveness could be adoptively transferred by draining lymph node cells. Although the timing and the incidence of the appearance of unresponsiveness was different between their results and ours, this might be due to the difference in the strain of rats used and that in the amount and the schedule of the low dose priming employed. In the present study, complete unresponsive state could be adoptively transferred to recipients with a 100% efficacy.

It was shown that adoptive transfer of either spleen cells or non-draining lymph node cells obtained 8 weeks after low dose priming have no or only marginal suppressive effect on the arthritogenic reaction. This suggests that suppressor cells are mainly harbouring in the lymph nodes draining the injected site of the adjuvant.

Certain incubation period was required for the generation of suppressor cells in the present experimental system. Similar to our findings, Watson & Collins (1980) reported the long incubation period for the development of DTH suppressor T cells. In contrast, arthritogenic reaction could be transferred by lymphoid cells obtained 8–11 days after the sensitization with mycobacterial adjuvant (Pearson & Wood, 1964).

We have found that rats primed with subarthritogenic dose of mycobacterial adjuvant could elicit severe arthritis after reinoculation with non-mycobacterial synthetic adjuvant, suggesting that low dose induced unresponsiveness is antigen specific. In contrast to our study, Kayashima *et al.* (1976, 1978) reported that, by using low responder strains of rats, poor susceptibility of these strains could be attributed to naturally existing, thymus derived cells. Thus, it is probable that major suppressor cells involved in the pathogenesis of adjuvant arthritis in low responder strain are non-specific naturally existing T cells, whereas those in high responder strain are antigen specifically induced T cells.

In terms of the susceptibility of suppressor cells to various immunosuppressive treatments such as irradiation or hydrocortisone administration, several different results have been reported (Tada *et al.*, 1971, Kayashima *et al.*, 1976, 1978, Yamaguchi & Kishimoto, 1978). Since pathogenesis of adjuvant arthritis seems to be not simply a cell-mediated response but rather the result of complex interaction of cell-mediated and humoral responses (Mackenzie *et al.*, 1978), more than one type of suppressor cells may be involved in the regulation of adjuvant arthritis.

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