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# A possible role of PMN in a casein-induced enhancement of PFC response to sheep erythrocytes in mice

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**Summary.** The effect of inflammation induced by sodium caseinate or aluminum hydroxide on the splenic plaque-forming cell (PFC) response to sheep red blood cells (SRBC) was studied in mice. Direct and indirect splenic PFC responses were enhanced when suboptimal SRBC doses ($3 \times 10^6$) were injected intraperitoneally (i.p.) within 9 hr of i.p. inflammatory stimulation; antigen administration 48 hr or more after such stimulation resulted in a slight suppression of the direct response. The inflammation had no effect on the secondary immune response, nor did intravenous antigen administration enhance the PFC response. Enhancement occurred when early (3 hr), casein-induced peritoneal exudate cells (PEC, consisting mostly of neutrophils) were adoptively transferred at the same time as antigen. Treatment of the 3-hr PEC with anti-Thy-1 and complement did not decrease their PFC-enhancing capability. Late (96-hr) PEC, consisting mostly of macrophages, manifested only a slight enhancing effect. We suggest that enhancement of the splenic PFC response in the presence of an ongoing inflammation, may be partially attributable to neutrophil function.

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## INTRODUCTION

Inflammatory and immune responses are respectively the prime examples of non-specific and specific mechanisms involved in the recognition and elimination of foreign substances. Usually, if a foreign antigenic substance is introduced into a tissue, both mechanisms come into play and their co-operation may be expected. Although the immune mechanism is known to support the function of the inflammatory response in various ways, the reciprocal situation in which inflammation may modulate the immune response has scarcely been investigated (Elves, 1972). Some workers have suggested that inflammation may have an enhancing effect on the immune response (Landsteiner & Jacobs, 1935; Friedlaender, Chisari & Baer, 1973; Kinnart, Mahieu & Geertruyden, 1979) and we reported previously that in the early stage of inflammation, inflammatory exudate exerts DNA synthesis-potentiating activity on lymphocytes (Yoshinaga, Nakamura & Hayashi, 1975).

Many adjuvant substances possess powerful phlogistic activities and their effect depends on the interval between the experimental introduction of antigen and the administration of the adjuvant. Usually, the adjuvant effect was observed when the antigen was introduced simultaneously or shortly after the injection of adjuvants (Dresser, 1968; Franzl & McMaster, 1968; Bradfield, Souhami & Addison, 1974; Turner & Higginbotham, 1977; Ghaffar & Sigel, 1978). These

intervals coincide with those during which we found the inflammatory exudate to manifest DNA synthesis-potentiating activity (Yoshinaga *et al.*, 1975).

In the present study we investigated the plaque-forming cell (PFC) response to sheep red blood cells (SRBC) injected into the inflamed peritoneal cavity of mice and analysed the role of inflammatory peritoneal exudate cells (PEC) in the modulation of the PFC response.

## MATERIALS AND METHODS

*Animals and immunization*

C3H/He and C57Bl/6N mice of either sex, 7–12 weeks of age, were used. In each set of experiments, siblings of the same sex were immunized intraperitoneally (i.p.) or intravenously (i.v.) with sheep red blood cells (SRBC) and 3–8 days later, splenic plaque-forming cells (PFC) were determined.

*Inflammation*

The mice were injected i.p. with 2 ml of 0·2% sodium caseinate (Yoshinaga *et al.*, 1975), 2 ml of 0·05% aluminum hydroxide gel (alum) in phosphate-buffered saline (PBS), or 2 ml of PBS. Alum was prepared by the conventional method (Garvery, Cremer & Susdorf, 1977) in the absence of antigen. Inflammatory PEC were collected 3 or 96 hr after casein injection by washing the peritoneal cavity with Hanks's balanced salt solution and washed three times with PBS.

*Anti-inflammatory drugs*

Acetyl salicilic acid (Shionogi Pharmaceutical Co., Osaka, Japan) was dissolved in carboxy methyl cellulose and administered *per os* (200 mg/kg body weight) 1 hr before the i.p. injection of casein (Vinegar, Truax & Selph, 1973). Indomethacin (Sigma Chemical Co., St Louis, Mo.) or phenylbutazone (Sigma Chemical Co.) was dissolved in 0·85% NaCl containing 0·09% Tween 80 and injected subcutaneously (s.c.) at the same time as an i.p. injection of casein (Goldstein, De Meo, Shemano & Beiler, 1966). Three hours after the casein injection, the animals were injected i.p. with $3 \times 10^6$ SRBC and the resulting splenic PFC was estimated after 4 days. The control animals were treated with vehicles without an anti-inflammatory drug.

*PFC assay*

Spleen cell suspensions were prepared from individual mice at appropriate intervals after immunization. Direct and indirect PFC in the cell suspension were developed by the conventional slide technique (Dresser, 1978). The PFC number per spleen was expressed as the mean ± SE (number of animals per group is given in each illustration).

*Anti-Thy-1 treatment of peritoneal exudate cells*

The 3-hr PEC from C3H/He mice were treated with AKR anti-Thy 1.2 antibody (Searle Diagnostic, High Wycombe) and rabbit complement (Cedarlane Lab. Ltd, Hornby, Ontario, Canada). For sham treatment, normal AKR serum was used instead of antiserum.

## RESULTS

### Effect of an ongoing inflammation on splenic PFC response to SRBC

The i.p. injection of sodium caseinate induced a mild, acute inflammation. As shown in Fig. 1, the injection of $3 \times 10^6$ SRBC into PBS-treated control mice resulted in the production of 3720 ± 652 PFC, irrespective of the interval between PBS and SRBC administration. The splenic PFC response was

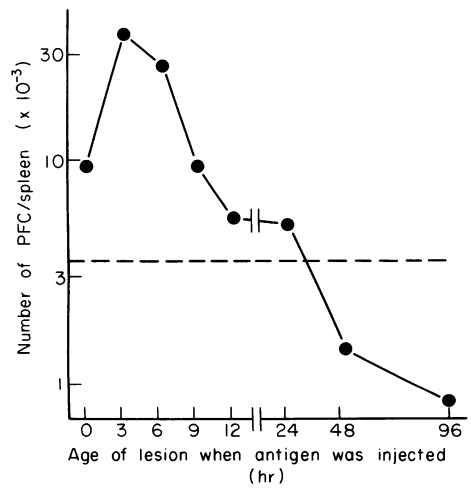


**Figure 1.** Direct splenic PFC response in C3H/He mice during a 96-hr observation period. At the indicated intervals after i.p. casein treatment, SRBC ($3 \times 10^6$) were injected i.p. and PFC per spleen was determined at 4 day after the SRBC. The result represents the mean of two mice, except the results at 3 and 96 hr. At 3 hr after casein, mean PFC of eight mice was 37,552 ± 1825. At 96 hr after casein, mean PFC of eight mice was 910 ± 120. In twenty-eight PBS-treated controls (----), the mean number of PFC (3720 ± 652) did not change irrespective of the interval between PBS and SRBC administration.

**Table 1.** Effect of casein-induced inflammation on splenic PFC response in C57Bl/6N and C3H/He mice

| Strain | Pretreated with | PFC per spleen | Enhancing index* |
|---|---|---|---|
| C57Bl/6N | PBS | 3746 ± 821 | — |
| | Casein | 22,902 ± 5100 | 6·1 |
| C3H/He | PBS | 3441 ± 449 | — |
| | Casein | 27,961 ± 7371 | 8·1 |

Casein or PBS was injected i.p. 3 hr before i.p. immunization with $3 \times 10^6$ SRBC; the direct splenic PFC response was estimated 4 days later. Mean ± SE, $n = 5$.

* PFC per spleen in casein-pretreated mice/PFC per spleen in PBS-pretreated mice.

obviously enhanced in mice which had received the antigen at the same time as, or within 9 hr of, casein administration. The highest PFC response during the 96-hr observation period was noted in mice sensitized 3 hr after casein injection. The PFC response of casein-treated mice was five to thirty-two times that of the PBS-treated mice; the mean was ten times. The enhancing effect of casein treatment was transient, it disappeared 24 hr postinjection and was replaced by slight PFC suppression after 48 hr. A similar enhancement of the splenic PFC response was observed on day 4 in C57Bl/6N mice that had been treated with $3 \times 10^6$ SRBC 3 hr after casein injection (Table 1).

To determine the antigen dose–response relationship, varying amounts of SRBC were injected i.p. 3 hr after casein or PBS treatment. Comparison of background PFC in the absence of SRBC administration revealed no significant difference between casein- and PBS-treated mice. As shown in Fig. 2, when the direct splenic PFC response was assessed 4 days after immunization, significant ($P < 0{\cdot}005$) enhancement was noted in casein-treated mice injected with antigen doses between $3 \times 10^6$ and $1 \times 10^7$; at $1 \times 10^8$, there was no significant difference from the controls. At $1 \times 10^6$ SRBC, the splenic PFC response in the control mice was very low and while PFC enhancement by casein-pretreatment was only slight, it was statistically significant ($P < 0{\cdot}025$). Furthermore, as shown in Fig. 3, when the splenic PFC response was assessed 4 days after the i.v. administration of various antigen doses, there was no difference between casein-treated and control mice.

Mice were pretreated with casein or PBS and 3 hr later, $3 \times 10^6$ SRBC were injected i.p. At different intervals after antigen administration, the direct splenic PFC response was determined. As shown in Fig. 4, the peak PFC response in control mice was observed on day 5, in casein-treated mice, it occurred somewhat earlier. In the latter animals, the splenic PFC response on days 3 and 4 was significantly (day 3, $P < 0{\cdot}005$; day 4, $P < 0{\cdot}001$) higher than in the controls. Although the number of PFC on day 5 was somewhat higher in casein-treated mice, this was not statistically significant.

In preliminary experiments, mice were injected i.p.

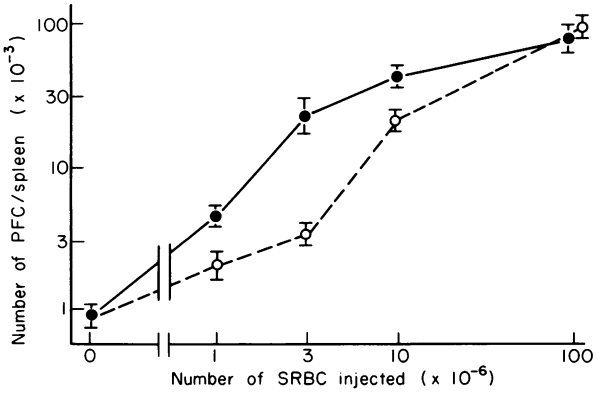


**Figure 2.** Dose–response relationship between SRBC and the direct splenic PFC response. Different antigen doses were injected (i.p.) 3 hr after the i.p. injection of casein (●——●) or PBS (o----o). The PFC response was determined at 4 days after the SRBC. Mean ± SE (ten animals per group).

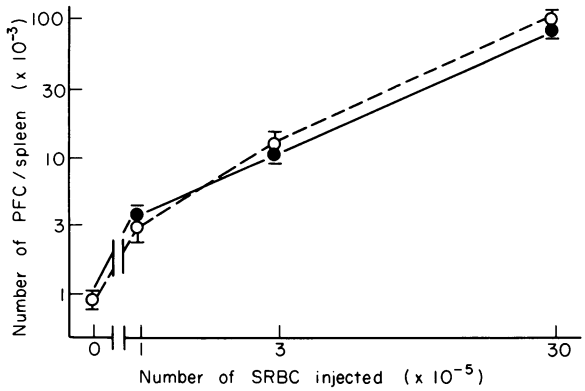


**Figure 3.** Direct splenic PFC response 4 days after i.v. administration of various SRBC doses. Mice were pretreated by i.p. injection of casein (●——●) or PBS (o----o) 3 hr before SRBC administration. Mean ± SE, $n = 4$.

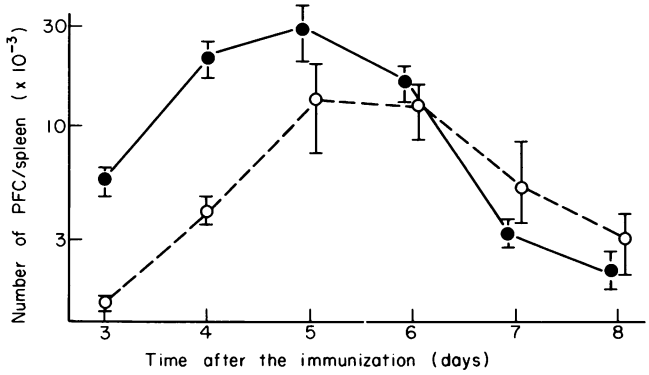


**Figure 4.** The kinetics of the direct splenic PFC response were estimated in C3H/He mice pretreated with casein (●——●) or control PBS (o----o). $3 \times 10^6$ SRBC were injected i.p. 3 hr after the casein or PBS. Mean ± SE; $n = 8$ on days 4–6; $n = 4$ on days 3, 7 and 8.

with casein or PBS and 3 hr later, they were immunized i.p. with $3 \times 10^6$ SRBC. The indirect splenic PFC response was assessed at days 3–7 post-antigen administration. Because in most mice, indirect PFC were hardly detectable on days 3 and 4 although a few indirect PFC were detected in some casein-treated mice (data not shown), the indirect PFC response was assessed on days 5 and 6. Control mice exhibited no indirect PFC response, while all casein-treated mice revealed a low but definite increase in indirect splenic PFC; enhancement was significant ($P < 0.05$) on day 6 (Table 2).

Mice were injected i.p. with a 2 ml suspension of non-antigenic alum and 3 hr later, they were immunized i.p. with $3 \times 10^6$ SRBC. The direct splenic PFC response was determined 4 days after antigen treatment. As shown in Table 3, the induction of inflammation by alum resulted in strong enhancement of the direct splenic PFC response. The range of PFC in alum-treated mice was from 38,072 to 86,935; in the controls it was from 246 to 4365.

To examine the effect of an ongoing inflammation on the secondary immune response, casein- or PBS-pretreated mice were immunized i.p. 3 hr later with $3 \times 10^6$ SRBC and 2 weeks thereafter, they were treated i.p. with a secondary antigen ($3 \times 10^6$–$3 \times 10^7$ SRBC). As shown in Fig. 5, 4 days after the second immunization, there was no significant differences in direct and indirect PFC responses between casein- and PBS-treated mice.

**Table 2.** Effect of casein-induced inflammation on indirect PFC response

| Experiment | PFC on day 5 in animals pretreated with | | PFC on day 6 in animals pretreated with | |
|---|---|---|---|---|
| | PBS | Casein | PBS | Casein |
| 1 | 0, 464 | 2095, 6552 | ND | ND |
| 2 | 1536 | 15,152 | 0 | 1436 |
| 3 | ND | ND | 0, 0 | 6311, 12,013 |
| 4 | ND | ND | 0, 0 | 900, 5400 |
| 5 | ND | ND | 0, 900 | 0, 1125 |

Casein or PBS was injected i.p. 3 hr before i.p. immunization with $3 \times 10^6$ SRBC. Indirect PFC per spleen were estimated on days 5 and 6.

Zero indicates that no indirect PFC were detected after counting $5 \times 10^5$ nucleated cells.

ND, not done.

In each set of experiments, siblings of the same sex were used. The individual PFC values in each set of experiments were given since the values were highly variable.

**Table 3.** Effect of alum-induced inflammation on PFC response

| Pretreated with | PFC per spleen | Enhancing index* |
|---|---|---|
| PBS | $3739 \pm 489$ | — |
| Alum | $63{,}567 \pm 11{,}225$ | 17 |

C3H/He mice were injected i.p. with alum or PBS 3 hr before i.p. immunization with $3 \times 10^6$ SRBC; the direct PFC response was assessed 4 days later. Mean $\pm$ SE, $n = 4$.

* PFC per spleen in alum-pretreated mice/PFC per spleen in PBS-pretreated mice.

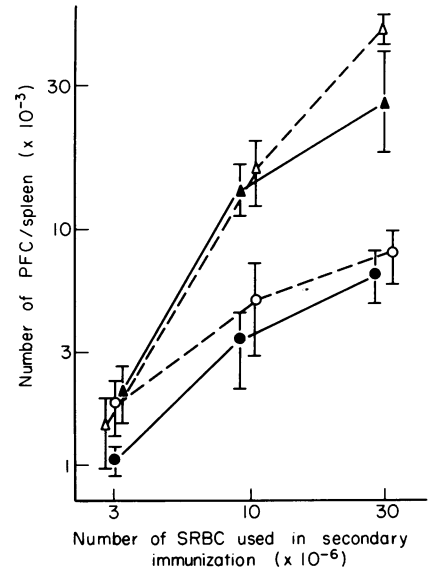


**Figure 5.** Effect of casein pretreatment on the secondary PFC response. C3H/He mice were pretreated with casein or PBS 3 hr before the first immunization with $3 \times 10^6$ SRBC. Two weeks thereafter, they received a secondary immunization with various SRBC doses. Direct and indirect PFC were estimated 4 days after the second immunization. Mean $\pm$ SE, $n = 4$. Direct (●——●) and indirect (▲——▲) PFC in casein-treated mice; direct (O----O) and indirect (△----△) PFC in control mice.

### Reproduction of the enhanced PFC response by adoptive transfer of early inflammatory exudate cells

At 3 and 96 hr after casein-, alum- or SRBC treatment, PEC were collected, counted and Giemsa-stained for leucocyte analysis. As shown in Table 4, 3 hr after

**Table 4.** Analysis of peritoneal exudate cells after i.p. stimulation with various agents

| Time after stimulation (hr) | Stimulation with | Number of PEC per mouse ($\times 10^{-6}$) | Analysis of PEC (%) | | | |
|---|---|---|---|---|---|---|
| | | | Neutrophils | Eosinophils | Lymphocytes | Macrophages |
| | None | 2·5 | 0 | 3·9 | 37·5 | 58·6 |
| 3 | Casein | 3·0 | 93·1 | 0·8 | 4·0 | 2·1 |
| | Alum | 4·2 | 84·1 | 0 | 11·9 | 4·0 |
| | SRBC | 3·4 | 5·2 | 0·1 | 24·7 | 70·0 |
| 96 | Casein | 4·0 | 0 | 0 | 25·7 | 74·3 |
| | Alum | 2·5 | 29·2 | 9·5 | 19·7 | 41·6 |
| | SRBC | 3·3 | 0 | 15·9 | 48·9 | 35·2 |

C3H mice were injected i.p. with 2 ml of 0·2% casein, 0·05% alum or $1{\cdot}5 \times 10^6$/ml SRBC. The resulting PEC were collected by washing the peritoneal cavity 3 or 96 hr later.

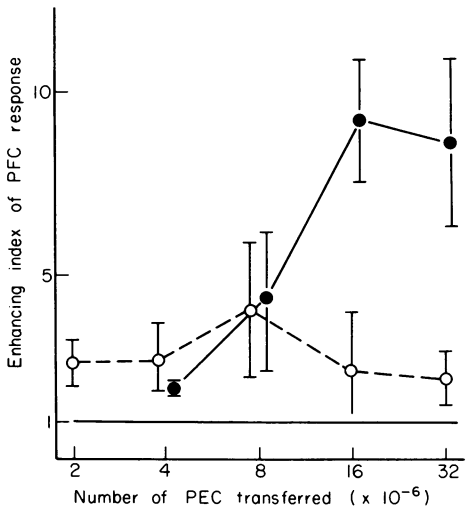


**Figure 6.** Effect of transferred inflammatory exudate cells on the direct PFC response. Casein-induced 3-hr (●——●) or 96-hr PEC (O----O) were transferred to the peritoneal cavity of sex-matched C3H/He mice together with $3 \times 10^6$ SRBC. Direct splenic PFC were estimated 4 days later. Mean ± SE of the enhancing index, $n = 6$. Enhancing index = [(PFC in animals injected with PEC and SRBC)/(PFC in animals injected with SRBC alone)]. Direct PFC in control mice injected with SRBC alone: 2427 ± 1052 per spleen, $n = 21$.

casein or alum stimulation, PEC consisted primarily of neutrophils; alum treatment resulted in a greater increase in total PEC than casein stimulation. The preponderance of neutrophil exudation persisted for approximately 9 hr in casein- and alum-treated mice (data not shown). At 3 hr after SRBC stimulation ($3 \times 10^6$), an increase in total PEC was noted; the dominant populations were macrophages (70%) and lymphocytes (24·7%); neutrophils constituted only 5·2% of the total population. At 96 hr after casein stimulation, PEC consisted of macrophages and lymphocytes; no neutrophils were detected. On the other hand, while the total number of PEC was lower 96 hr after alum treatment, neutrophils and eosinophils constituted 29·2% and 9·5%, respectively, of the PEC population. At 3 and 96 hr after the combined injection of 2 ml 0·2% casein and $3 \times 10^6$ SRBC, the total number of PEC was almost equal to the sum of these cells obtained upon the individual injection of these agents (data not shown).

To test whether PEC affect the immune response, varying amounts of inflammatory exudate cells obtained 3 and 96 hr after casein injection were simultaneously injected with $3 \times 10^6$ SRBC and the splenic PFC response was determined 4 days later. As shown in Fig. 6, the simultaneous injection of 3-hr PEC resulted in a dose-dependent enhancement of the splenic PFC response. On the other hand, 96-hr PEC enhanced the PFC response only slightly and there was no dose–response relationship.

As the casein-induced 3-hr PEC contained 4% lymphocytes, we examined whether they were responsible for the observed enhancement of the splenic PFC response. Although the number of T lymphocytes was small, they may play a key role in the induction of such enhancement. T lymphocytes were removed from 3-hr PEC by treatment with anti-Thy-1 antibody and rabbit complement; in the control, PEC were treated with normal AKR serum and complement. After three times washings of the T-lymphocyte-depleted PEC, they were i.p. transferred in the presence of $3 \times 10^6$ SRBC and the direct splenic PFC response was determined 4 days later. As shown in Table 5, in the absence of T lymphocytes, the PFC-enhancing ability of 3-hr PEC was not decreased, rather, T-cell-depleted PEC enhanced the direct splenic PFC response.

### Effect of the systemic administration of anti-inflammatory drugs on casein-induced enhancement of splenic PFC response

Mice were systemically administered with acetyl salicilic acid, indomethacin or phenylbutazone. Antigen ($3 \times 10^6$ SRBC) was administered 3 hr after an i.p.

**Table 5.** Effect of transferred anti-Thy-1 treated PEC on the direct PFC response

| PEC treatment | Number of PEC transferred | PFC per spleen |
|---|---|---|
| | None | 2904 |
| Normal AKR serum plus C | $8 \times 10^6$ | 14,813 |
| | $16 \times 10^6$ | 30,783 |
| Anti-Thy 1.2 plus C | $8 \times 10^6$ | 47,053 |
| | $16 \times 10^6$ | 135,326 |

Casein-induced 3-hr PEC from C3H/He mice were treated with AKR anti-Thy 1.2 antibody (diluted 1:32) plus rabbit complement (C, diluted 1:10), or with normal AKR serum plus C and transferred to sex-matched siblings together with $3 \times 10^6$ SRBC. The direct PFC response was estimated 4 days later. Results represent the mean of duplicate experiments.

**Table 6.** Effect of anti-inflammatory drugs on casein-induced enhancement of splenic PFC response

| Casein pretreatment* | Drug administration | Number of PMN† exuded ($\times 10^{-6}$) | PFC per spleen‡ |
|---|---|---|---|
| − | — | 0 | 3240 ± 522 |
| + | — | 3·2 | 28,360 ± 935 |
| + | CMC§ | 3·1 | 31,450 ± 6004 |
| + | Tween 80¶ | 3·2 | 33,580 ± 2432 |
| + | Acetyl salicilic acid | 3·2 | 36,080 ± 5661 |
| + | Indomethacin | 3·2 | 47,940 ± 5310 |
| + | Phenyl butazone | 3·2 | 42,790 ± 14,166 |

* Two millilitres of 0·2% casein was injected i.p. 3 hr before the i.p. SRBC ($3 \times 10^6$).

† Number of i.p. exuded PMN was estimated 3 hr after the casein injection. Values were means of two animals.

‡ Direct PFC per spleen was determined 4 days after SRBC injection. Results represent the mean with SE (number of animals per group was six).

§ One-fifth of a millilitre of 0·5% carboxymethyl cellulose (vehicle for acetyl salicilic acid) was administered *per os* 1 hr before the i.p. injection of casein.

¶ One-fifth of a millilitre of 0·09% Tween 80 in physiological saline (vehicle for indomethacin and phenylbutazone) was injected subcutaneously at the same time as an injection of i.p. casein.

injection of casein and 4 days later, splenic PFC was determined. PMN exudation was recorded 3 hr after the casein injection in both the anti-inflammatory drug administered groups and the control groups.

As shown in Table 6, none of these anti-inflammatory drugs inhibited the casein-induced PFC response. Slight PFC-enhancement was observed in the indomethacin and phenylbutazone-administered groups, though it was not statistically significant. The number of exuded PMNs 3 hr after i.p. casein did not show any reduction from those of the control groups.

## DISCUSSION

The present investigation demonstrated that existing inflammation has dual modulating effects on the primary anti-SRBC PFC response. Antigen administration in the early inflammatory stage induced a strong enhancement of the immune response while its administration during the later course of inflammation resulted in a normal, or slightly depressed, PFC response (Fig. 1).

Although casein is not generally considered to be an adjuvant, its ability to enhance the immune response to suboptimal SRBC doses is suggestive of its adjuvant effects. The interval between antigen introduction and adjuvant administration plays a role in the level of antibody production. Previous investigations have shown that antigen administration at relatively short intervals after adjuvant treatment results in an enhanced antibody response; after longer intervals, the response is unaltered or suppressed (Franzl & McMaster, 1968; Unanue, Askonas & Allison, 1969; Bradfield *et al.*, 1974; Turner & Higginbotham, 1977). These results coincide with our present finding. On the other hand, after *Corynebacterium parvum* (Warr & Sljivic, 1974) and silica (Pernis & Paronetto, 1962) stimulation, the interjection of a prolonged interval before antigen administration, brings about an enhanced immune response.

The modulation of the immune response induced by the successive injection of two different substances is a complex phenomenon which may be explicable in several ways. Upon the successive injection of two antigens, the antibody response to the second antigen is enhanced—or suppressed—depending on the time that has elapsed since the introduction of the first antigen. This phenomenon has been attributed to the activity of antigenically non-specific helper or suppressor T lymphocytes which are activated by the first antigen (Dresser, 1968; Möller & Sjöberg, 1970; Depelcin & Huygen, 1977). However, this explanation is inadequate with respect to our present results,

because splenic PFC enhancement was induced by alum, a non-antigenic phlogistic substance (Table 3), as well as by the transfer of T-lymphocyte depleted 3-hr PEC (Table 5).

The relationship between macrophages, especially activated macrophages, and adjuvants has been established. Endotoxic lipopolysaccharide (Boehame & Dubos, 1958; Rowley, 1960), Freund's complete adjuvant (Laufer, Tal & Behar, 1959), *Corynebacterium* (Howard, Christie & Scott, 1973), and oligonucleotides (Braun & Firshein, 1967) increase the phagocytic activity and proliferation of mononuclear phagocytes. Unanue *et al.* (1969) have reported that the macrophagic uptake of antigens and adjuvants results in an enhanced immune response when these cells are adoptively transferred to normal mice.

Our present results do not preclude the possibility that the injection of casein or alum resulted in the activation of the mononuclear phagocyte system. However, the immune response was slightly suppressed, rather than enhanced, when the antigen was injected into the inflamed peritoneal cavity where macrophage exudation was predominant (Table 4). Furthermore, the adoptive transfer of 96-hr casein-induced PEC, which are primarily constituted of macrophages (Table 4), did not result in immune response enhancement comparable to that when neutrophils were adoptively transferred (Fig. 6). In fact, it is difficult to correlate murine haemolysin production with macrophage activation following their stimulation with bacterial lipopolysaccharide (Franzl *et al.*, 1968). These considerations appear to speak against the macrophage activation hypothesis.

Others (Diamantstein, Meinhold & Wagner, 1971; Souhami, 1972; Bradfield *et al.*, 1974) have attributed the action of adjuvants to an alteration in the antigen distribution, especially the high antigen uptake by the spleen following the injection of adjuvants. Thomas, Singer, Tieney, Folch & MacSween (1976) reported that cirrhotic rats manifested an enhanced immune response and altered antigen distribution, i.e. decreased hepatic and increased splenic uptake. Conversely, estrogen-treated mice showed a decrease in the immune response and the splenic uptake of antigen (Sljivic, Clark & Warr, 1975). These observations appear to support the conception that the altered antigen distribution plays a role in immune modulation. The present investigation does not rule out the possibility that altered antigen distribution may induce the enhanced immune response in mice with intraperitoneal inflammation or an adoptive transfer of neutrophils. A similar enhancement of the immune response has been observed in operated rats, although no correlation between antibody synthesis and increased splenic antigen levels was observed (Kinnart *et al.*, 1979). Furthermore, in mice treated with T-dependent and T-independent antigens and *C. parvum*, there was no correlation between the antigen distribution and the immune response (Warr & Sljivic, 1974). These considerations make it impossible to attribute the increased immune response solely to a large antigen uptake by the spleen.

Three non-steroidal anti-inflammatory drugs were chosen for evaluating a reversing effect on the casein-induced enhancement of PFC response. But, all of these representative anti-inflammatory drugs failed to reverse the enhanced PFC response. These drugs also failed to suppress the PMN emigration at 3 hr after casein injection. At present, no effective anti-inflammatory drug, except anti-inflammatory steroids, is known to inhibit the PMN emigration *in vivo* (DiRosa, 1979). The drugs used here are known to have a good inhibitory ability on oedema formation and mononuclear cell exudation (DiRosa, Papadimitriou & Willoughby, 1971). Therefore, this failure of the reversing effect on enhanced PFC response seemed not to negate a role of PMN in the enhancement, but suggests that the oedema formation or mononuclear cell emigration may not be related to the PFC enhancement.

The role of neutrophils in adjuvant action has received little attention to date. In view of our previous findings which revealed that neutrophils, stimulated by the existence of an inflammation, release a certain lymphocyte-activating substance(s) (Yoshinaga *et al.*, 1975; Nakamura, Yoshinaga & Hayashi, 1976; Yoshinaga, Nishime, Nakamura & Goto, 1980), investigations are under way to examine a role of inflammatory soluble substance in the modulation of humoral immune response.

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## REFERENCES


BOEHME D. & DUBOS R.J. (1958) The effect of bacterial constituents on the resistance of mice to heterologous infection and on the activity of their reticulo-endothelial system. *J. exp. Med.* **107,** 523.

BRADFIELD J.W.B., SOUHAMI R.L. & ADDISON I.E. (1974) The mechanism of the adjuvant effect of dextran sulphate. *Immunology*, **26,** 383.

BRAUN W. & FIRSHEIN W. (1967) Biodynamic effects of oligonucleotides. *Bact. Rev.* **31,** 83.

DEPELCIN A. & HUYGEN K. (1977) Interference between two immune responses. Modification of the kinetics of the mouse response to SRBC by responses to heterologous RBC. *Ann. Immunol.* **128C,** 633.

DIAMANTSTEIN T., MEINHOLD H. & WAGNER B. (1971) Stimulation of humoral antibody formation by polyanions. V. Relationship between enhancement of sheep red blood cell uptake by the spleen and adjuvant action of dextran sulphate. *Europ. J. Immunol.* **1,** 429.

DIROSA M. (1979) Inhibition of cell migration *in vivo* and granuloma formation. In: *Anti-inflammatory drugs* (Ed. by J.R. Vane & S.H. Ferreira), p. 223. Springer-Verlag, Heidelberg.

DIROSA M., PAPADIMITRIOU J.M. & WILLOUGHBY D.A. (1971) A histopathological and pharmacological analysis of the mode of action of non-steroidal anti-inflammatory drugs. *J. Pathol.* **105,** 239.

DRESSER D.W. (1968) An assay for adjuvanticity. *Clin. exp. Immunol.* **3,** 877.

DRESSER D.W. (1978) Assays for immunoglobulin-secreting cells. In: *Handbook of Experimental Immunology*, 3rd edn (Ed. by D.M. Weir), ch. 28, p. 2. Blackwell Scientific Publications, Oxford.

ELVES M.W. (1972) *The lymphocytes*, 2nd edn. Lloyd-Luke, London.

FRANZL R.E. & MCMASTER P.D. (1968) The primary immune response in mice. I. The enhancement and suppression of hemolysin production by a bacterial endotoxin. *J. exp. Med.* **107,** 1087.

FRIEDLAENDER M.H., CHISARI F.V. & BAER H. (1973) The role of the inflammatory response of skin and lymph nodes in the induction of sensitization to simple chemicals. *J. Immunol.* **111,** 164.

GARVERY J.S., CREMER N.E. & SUSSDORF D.H. (1977) *Methods in Immunology*, 3rd edn, p. 185. W.A. Benjamin Inc., London.

GHAFFAR A. & SIGEL M.M. (1978) Immunomodulation by *Corynebacterium parvum*. I. Variable effects on anti-sheep erythrocyte antibody responses. *Immunology*, **35,** 685.

GOLDSTEIN S., DEMEO R., SHEMANO I. & BEILER J.M. (1966) A method for differentiating non-specific irritants from anti-inflammatory agents using the carageenin abscess test. *Proc. Soc. exp. Biol. Med.* **123,** 712.

HOWARD J.G., CHRISTIE G.H. & SCOTT M.T. (1973) Biological effects of *Corynebacterium parvum*. IV. Adjuvant and inhibitory activities on B lymphocytes. *Cell. Immunol.* **7,** 290.

KINNART P., MAHIEU A. & VAN GEETRUYDEN N. (1979) Kinetics of humoral responsiveness and antigenic distribution in operated rats. *Immunology*, **38,** 195.

LANDSTEINER K. & JACOBS J.L. (1935) Studies on sensitization of animals with simple chemical compounds. *J. exp. Med.* **61,** 643.

LAUFER A., TAL C. & BEHAR A.J. (1959) Effect of adjuvants (Freund's type) and its components on the organs of various animal species. *Br. J. exp. Pathol.* **40,** 1.

MÖLLER G. & SJÖBERG O. (1970) Effect antigenic competition on antigen-sensitive cells and on adoptively transferred immunocompetent cells. *Cell. Immunol.* **1,** 110.

NAKAMURA S., YOSHINAGA M. & HAYASHI H. (1976) Interaction between lymphocytes and inflammatory exudate cells. II. A proteolytic enzyme released by PMN as a possible mediator for enhancement of thymocyte response. *J. Immunol.* **117,** 1.

PERNIS B. & PARONETTO F. (1962) Adjuvant effect of silica (tridimite) on antibody production. *Proc. Soc. exp. Biol. Med.* **110,** 390.

ROWLEY D. (1960) The role of opsonins in non-specific immunity. *J. exp. Med.* **111,** 137.

SLJIVIC V.S., CLARK D.W. & WARR G.W. (1975) Effects of oestrogens and pregnancy on the distribution of sheep erythrocytes and the antibody response in mice. *Clin. exp. Immunol.* **20,** 179.

SOUHAMI R.L. (1972) The effect of colloidal carbon on the organ distribution of sheep red blood cells and the immune response. *Immunology*, **22,** 685.

THOMAS H.C., SINGER C.R.J., TILNEY N.L., FOLCH H. & MACSWEEN R.N. (1976) The immune response in cirrhotic rats. Antigen distribution, humoral immunity, cell-mediated immunity and splenic suppressor cell activity. *Clin. exp. Immunol.* **26,** 574.

TURNER E.V. & HIGGINBOTHAM R.D. (1977) Carageenin induced alterations of antigen distribution and immune responses in mice. *J. reticuloendeth. Soc.* **22,** 545.

UNANUE E.R., ASKONAS B.A. & ALLISON A.C. (1969) A role of macrophages in the stimulation of immune responses by adjuvants. *J. Immunol.* **103,** 71.

VINEGAR R., TRUAX J.F. & SELPH J.L. (1973) Some quantitative temporal characteristics of carageenin-induced pleurisy in the rat. *Proc. Soc. exp. Biol. Med.* **143,** 711.

WARR G.W. & SLJIVIC V.S. (1974) Enhancement and depression of the antibody response in mice caused by *Corynebacterium parvum*. *Clin. exp. Immunol.* **17,** 519.

YOSHINAGA M., NAKAMURA S. & HAYASHI H. (1975) Interaction between lymphocytes and inflammatory exudate cells. I. Enhancement of thymocyte response to PHA by product(s) of polymorphonuclear leukocytes and macrophages. *J. Immunol.* **115,** 533.

YOSHINAGA M., NISHIME K., NAKAMURA S. & GOTO F. (1980) A PMN-derived factor that enhances DNA synthesis in PHA or antigen-stimulated lymphocytes. *J. Immunol.* **124,** 94.