STUDIES ON THE PATHOGENESIS OF FEVER

XX. Suppression and Regeneration of Pyrogen-Producing Capacity of Exudate Granulocytes*

By S. FAI CHEUK,‡ Ph.D., HELMUT H. HAHN§, M.D., DOUGLAS M. MOORE‖, DONALD N. KRAUSE¶, PETER A. TOMASULO**, M.D., and W. BARRY WOOD, Jr., M.D.

(From the Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205)

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In a preceding paper (1) evidence was presented that blood leukocytes, which contain less pyrogen than *exudate* granulocytes (2) and release less when deprived of K^+ (2, 3), can be "activated" to behave like exudate leukocytes, if incubated in acute exudate fluid or in media containing small amounts of endotoxin. The activation process was shown to involve the synthesis of essential protein, believed to be either an inactive precursor of the pyrogen molecule (propyrogen), or enzyme(s) involved in the conversion of propyrogen to pyrogen.

The present studies deal with the conversion of exudate granulocytes to a refractory or *suppressed* state by incubation in plasma or serum. Included also is a study of the manner in which exudate granulocytes, depleted of pyrogen by repeated incubations in K-free saline, recover their capacity to produce pyrogen when incubated in serum.

Materials and Methods

All methods and reagents were as previously described (1, 4, 5) unless specifically stated in the tables or text.[1]

The concentration of cells suspended in all media was 3.5×10^7 per ml.

Incubations were at 37°C unless otherwise indicated.

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‡ Present address: New England Aquarium, Boston, Massachusetts.

§ Present address: Trudeau Institute, Saranac Lake, New York.

‖ Predoctoral trainee, United States Public Health Service Training Grant 5 T01 GM 0039.

¶ Present address: University of Maryland School of Medicine, Baltimore, Maryland.

** Present address: Department of Medicine, Johns Hopkins Hospital, Baltimore, Maryland.

[1] Peritoneal exudates were induced (5) with a single lot of shellfish glycogen (Mann Research Laboratories, Inc., New York). A new lot, used in later confirmatory experiments (not reported), induced a more intense exudate containing "superactivated" (1) granulocytes,

RESULTS

A. *Suppression.*—

Suppression of exudate granulocytes by incubation in heparinized plasma or serum: Exudate granulocytes are suppressed by incubation in plasma or serum, in the sense that they release less pyrogen than untreated exudate cells when transferred to 0.15 M NaCl and incubated for 1 hr (Table I). The primary effect

TABLE I

Suppression of Exudate Leukocytes by Incubation in Heparinized Plasma or Serum

Suppressive medium	Incubation temperature	Incubation time	Average release of pyrogen in 0.15 M NaCl (FI_{120})*
	°C	hr	
Plasma	—	0	10.9 (± 0.7) (12)
Plasma	37	2	0.4 (± 0.2) (12)
Plasma	0	2	10.2 (± 1.2) (12)
Serum	—	0	10.6 (± 0.7) (9)
Serum	37	2	3.5 (± 1.0) (9)
Serum	0	2	11.5 (± 1.3) (9)

* From 3.5×10^7 cells incubated for 1 hr at 37°C; FI_{120} = average response of number of rabbits indicated in last parenthesis ± standard error of mean.

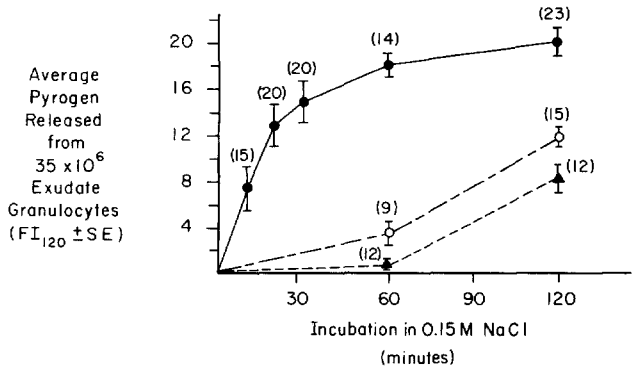

FIG. 1. Comparison of rates of average pyrogen released from untreated (●——●), serum-suppressed (○---○), and plasma-suppressed (▲--- ▲) exudate cells incubated (37°C) in 0.15 M NaCl. Data for untreated cells calculated from reference 6. The number of assays performed at each point is indicated in parenthesis, and the standard error of the mean (SE) is shown by the vertical bar running through the point. The cells were treated as described in Table I.

Footnote 1 (continued):
which were only minimally suppressed by incubation in plasma. When the concentration of injected new glycogen was reduced from 0.2% (5) to 0.02% the cells were consistently suppressed by plasma incubation, as in the original experiments.

of the suppressive process, which is temperature dependent (Table I, lines 2, 3, 5, and 6), is to slow the release of pyrogen, particularly during the first hour (Fig. 1). It will be noted that serum suppresses the cells less completely than does plasma (Table I, lines 2 and 5, and Fig. 1).

Identification of suppressive factor: The data summarized in Table II indicate that the principal suppressive factor in plasma is Ca^{++}. This conclusion is

TABLE II

Evidence that Principal Suppressive Factor in Plasma is Ca^{++}

Suppressive medium	Incubation	Average release of pyrogen in 0.5 M NaCl (FI_{120})*
	hr	
None (control)	0	11.5 (± 0.5) (12)
Plasma‡ + EDTA§	2	10.4 (± 0.9) (9)
" + EDTA-Ca§	2	1.0 (± 0.3) (9)
Plasma‡ dialyzed‖ vs. Na**	2	12.5 (± 0.9) (12)
" " " Na-K‡‡	2	10.5 (± 1.3) (12)
" " " Na-Ca§§	2	1.3 (± 0.4) (12)
" " " Na-K-Ca	2	0.7 (± 0.3) (12)
Saline (Na-K)	2	10.9 (± 1.7) (3)
" (Na-K-Ca 2 mM)	2	3.4 (± 0.5) (3)
" (Na-K-Ca 4 mM)	2	0.7 (± 0.2) (3)
" (Na-K-Mg 4 mM)	2	11.7 (± 1.4) (3)
" (Na-K-Sr 4 mM)	2	10.6 (± 0.2) (3)

* As in Table I.

‡ Heparinized.

§ 5 mM.

‖ In volumes of 10 ml against 600 ml of electrolyte solution changed four times during 24 hr of dialysis at 4°C.

** 150 mM NaCl.

‡‡ 5 mM KCl.

§§ 2 mM $CaCl_2$.

substantiated by the pronounced suppressive effect of Ringer's solution containing physiological concentrations of Ca^{++} (Table II, line 10). Neither Mg^{++} nor Sr^{++} can be substituted for the Ca^{++} (lines 11 and 12).

Kinetics of suppression: Suppression by either plasma or Ringer's solution is a relatively slow process requiring at least 2 hr incubation at 37°C (Fig. 2). Suppression also tends to prevent the release of cellular lysozyme (3).[2]

[2] S. F. Cheuk and W. B. Wood, Jr. Unpublished observations.

Relation of cellular Ca to suppression: The data summarized in Table III suggest that suppression involves uptake of Ca by the cells.

Reversibility of suppression: Repeated washing of plasma-suppressed cells in the cold with buffered sucrose that is relatively low in Ca^{++} (0.1 mM) causes them to be "desuppressed" (Table IV).

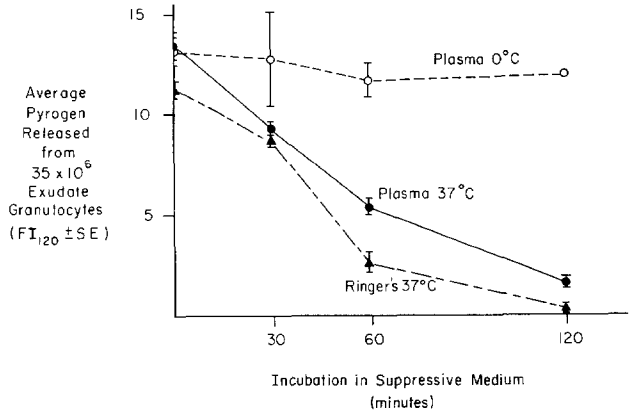

FIG. 2. Kinetics of suppressive process. On the abscissa is plotted the time that the exudate granulocytes were exposed to plasma at 4°C (O---O), plasma at 37°C (●——●), and Ringer's solution at 37°C (▲--- ▲) before being transferred to 0.15 M NaCl. On the ordinate is plotted the average amount of pyrogen released by transferred cells during incubation (37°C) for 1 hr in the saline medium. The value at each point represents the average fever response of three rabbits ± the standard error of the mean (indicated by vertical bar).

TABLE III

Effect of Suppression on Ca Content of Cells

Treatment of cells	Average Ca content*		
	Before	After	Difference
Plasma‡	5.5 (± 0.9)	7.5 (± 1.2)	+2.0 (± 1.0)
Ringer's solution§	4.2 (± 0.2)	5.0 (± 0.2)	+0.8 (± 0.1)

* Microequivalents (μEq) of total Ca per 3.5 × 10^8 cells measured by the murexide method of Walser (8).

‡ Incubated for 2 hr in heparinized plasma.

§ Incubated for 2 hr in Ringer's solution (150 mM NaCl, 5 mM KCl, 4 mM $CaCl_2$).

B. *Regeneration.*—

Exhaustion and regeneration of pyrogen production: When exudate granulocytes are repeatedly incubated in 0.15 M NaCl, they seem to become depleted

of pyrogen and release very little into the medium (Table V, line 1). If they are incubated in serum after the first incubation in saline, however, much of their pyrogen-producing capacity is restored (line 2). Neither incubation in heparinized plasma (line 4) nor exposure to serum in the cold (line 3) has this effect.

TABLE IV

Desuppression of Plasma-Suppressed Exudate Granulocytes

Treatment of cells	Average release of pyrogen in 0.15 M NaCl (FI_{120} ±)*
Plasma‡, no incubation (control)	7.4 (±0.5)
Plasma, incubation 2 hr	0 (±0)
Plasma, incubation 2 hr, repeated washing§ at 4°C	8.6 (±1.1)

* As in Table I.

‡ Heparinized.

§ Three times in 3 ml of buffered sucrose containing 2.7×10^{-2} M sucrose, 1×10^{-4} M $CaCl_2$, 2×10^{-3} M NaH_2PO_4, 3×10^{-3} M Na_2HPO_4 (pH 7.0).

TABLE V

Regenerating Effect of Serum on Pyrogen-Producing Capacity of Exudate Granulocytes

Incubation* sequence	Average release of pyrogen (FI_{120})‡ during:		
	NaCl-1	NaCl-2	NaCl-3
NaCl → NaCl → NaCl	12.94 (±0.79) (32)	8.26 (±1.21) (17)	1.78 (±0.61) (16)
NaCl → *Serum* → NaCl → NaCl	" " "	11.71 (±1.11) (37)	6.06 (±1.10) (13)
NaCl → *Serum*-0° → NaCl → NaCl	" " "	7.44 (±1.15) (14)	3.32 (±0.65) (6)
NaCl → *Plasma*§ → NaCl → NaCl	" " "	6.34 (±1.55) (8)	—

* 2 hr.

‡ As in Table I, (*).

§ Heparinized.

Blocking of regeneration with puromycin: The regeneration of pyrogen-producing capacity induced by incubating the cells in serum is blocked by appropriate concentrations of puromycin (Table VI). These concentrations of puromycin do not impair the release of pyrogen in the NaCl medium (7). It is concluded, therefore, that the regeneration process involves protein synthesis, and thus resembles the activation process (1).[3]

[3] Comparison of the data in Table VI with that in Tables X and XI of reference (1) suggests that the regenerative process is a little less sensitive to puromycin than the activation process. That there should be a slight difference is not surprising, since the conditions of the two sets of experiments are not identical.

TABLE VI

Blocking Action of Puromycin (P) on Regenerating Effect of Serum

Incubation* sequence	Average release of pyrogen (FI_{120})‡ during NaCl-2§
NaCl → Serum → NaCl	11.71 (±1.11) (37)
NaCl → Serum + P (2×10^{-4} M) → NaCl	10.44 (±1.96) (7)
NaCl → Serum + P (1×10^{-3} M) → NaCl	6.77 (±1.22) (8)

* 2 hr.

‡ As in Table I, (*).

§ As in Table V.

DISCUSSION

The terms "suppression" and "activation" used in this and a preceding report (1), refer to the ability of granulocytes to generate endogenous pyrogen when incubated in 0.15 M NaCl. Blood leukocytes release very little pyrogen under these conditions (2) and therefore, by definition, are *unactivated.* Exudate granulocytes under the same conditions generate large amounts of pyrogen (3) and hence are *activated.*

The ability of activated exudate granulocytes to release pyrogen in 0.15 M NaCl may also be suppressed by previous incubation in plasma, serum, or Ringer's solution. This suppressive effect involves the action of Ca^{++}. The divalent calcium ions presumably affect the cell membranes (9) and render them unresponsive to the K^+ deprivation stimulus (3). The unresponsiveness of the membranes may be due to the formation of additional Ca^{++} ligands in their matrix (10). In keeping with this hypothesis are the observations (*a*) that suppression seems to be associated with a cellular uptake of Ca, (*b*) that desuppression can be achieved by merely washing the cells repeatedly in Ca-poor sucrose solution, and (*c*) that suppression diminishes the leakage of proteins other than pyrogen (e.g. lysozyme) from K^+-deprived cells.[2]

A distinction must be made, however, between suppressed exudate granulocytes and unactivated blood cells. Both are unresponsive to the K^+ deprivation stimulus, but for different reasons. Whereas the membranes of the suppressed exudate cells are apparently unresponsive because of assimilation of additional Ca^{++}, the unactivated blood cells lack an essential protein (1). Furthermore, unactivated blood cells cannot be activated by removal of Ca^{++}, even with chelating agents like ethylenediaminetetraacetate (EDTA).[2, 4]

Regeneration of pyrogen production in pyrogen-depleted cells also requires protein synthesis and otherwise resembles the activation process (1). The finding that incubation in serum, but not in plasma, regenerates such cells is

[4] J. D. Morton and W. B. Wood, Jr. Unpublished observations.

in keeping with the superiority of plasma over serum as a suppressive agent (Table I), and suggests that serum, like acute exudate fluid (1), may contain an activator.

SUMMARY

Suppression of the pyrogen-producing capacity of exudate granulocytes results from incubation of the cells in plasma, serum, or Ringer's solution. When transferred in this state and incubated in isotonic NaCl, the cells release much less pyrogen than untreated exudate cells. The suppressive effect is reversible and appears to involve the cellular uptake of calcium ions.

In contrast, regeneration of pyrogen-producing capacity in depleted exudate cells occurs only when the cells are incubated in serum. The process resembles activation and requires the cellular synthesis of protein.

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