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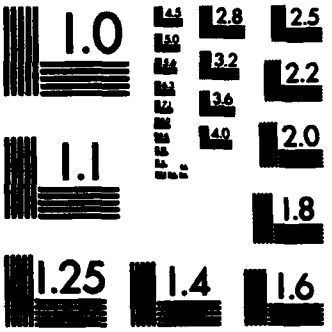

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The PFC response of SJL and BALB/c mice to sheep erythrocytes as well as to a polysaccharide antigen is enhanced by intravenous injection of 0.05 ml normal mouse or human serum but not by plasma. This is particularly evident in mice which have had an injection of an immunosuppressive agent along with the antigen. The factor responsible for this effect is readily absorbed by spleen cells from normal but not from athymic mice. The presence of proteolytic enzyme inhibitors during clotting prevents the appearance of this factor

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20. ABSTRACT(continued)

in serum. On the other hand, addition of thrombin to isolated platelets causes the secretion of this factor into the supernatant. Thus, a connection between platelets and the immune system is revealed by these findings.

The initial studies on the effect of various adjuvants on the immune response to pneumococcal polysaccharide types 3 and 14 have shown so far that a direct B cell activating agent, such as E. coli endotoxin, causes only a very moderate enhancement of the primary 19S antibody response and not sufficient antibody production to cause protective immunity, and that muramyl dipeptide (MDP) has even less effect, whether administered in an emulsion with the polysaccharide or in soluble form.

Effect Of Adjuvants On Response To Pneumococcal Polysaccharide Injected Intraperitoneally

Experiments were initiated on the effect of adjuvants on the response to i.p. and subcutaneous (footpad) injected pnuemococcal polysaccharide (Pn.ps.). The antibody response was evaluated by plaque forming cells (PFC) in spleen and draining (brachial) lymph node, antibody titers and by resistance to challenge with viable pneumococci of the same strain as the polysaccharide used for immunization.

Three polysaccharides (Pn.ps. 3,14 and 19) were examined for their ability to induce PFC responses, serum antibody and protective immunity in BALB/c and SJL mice after i.p. injection. BALB/c mice responded to Pn.ps. 3 and 14, but not to Pn.ps 19. SJL mice also failed to respond to type 19, and showed lower responses to type 14 than BALB/c mice. Various adjuvants, including B. pertussis, MDP, and LPS were used in attempts to induce responses to Pn.ps. 19, without any results. In contrast, the PFC response to Pn.ps. 3 and 14 was enhanced by i.p. injection of LPS, B. pertussis and 8-mercaptoguanosine, but not by MDP and polyAU, not even when MDP was given together with the polysaccharide in an emulsion.

The response to Pn.ps injected into the footpad was so far not detectable by PFC in the draining lymph node although serum antibody titers were higher than in controls. This is now under further examination.

Whole heat-killed pneumococci types 14 and 19 were also used for the immunization of BALB/c mice. In the case of type 14, they induced better responses than polysaccharide alone, but a response to type 19 was still not obtained. Already on days 4-5 after a single injection of 1.2×10^9 killed whole pneumococci type 14 + LPS i.p., the spleen contained 4,500 - 5,000 PFC.

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Platelet-Derived Immunoregulatory Activity

When SJL mice are injected intravenously (iv) with γ-irradiated syngeneic lymphoma (γ-RCS) cells at the same time as sheep erythrocytes (SRBC) a marked suppression of the ensuing plaque forming cell (PFC) response in the spleen is observed [Katz et al., J. Natl. Cancer Inst. 72:125, 1984]. This suppression has been interpreted as due to the strong T cell proliferation [Hayama et al., J. Natl. Cancer Inst. 72:321, 1984], IL-2 [Hayama et al., Cell. Immunol. 79:134, 1983] and IFN-γ [Ponzio et al., J. Natl. Cancer Inst. 72:311, 1984] production induced by γ-RCS both in vivo and in vitro. Cyclophosphamide pretreatment prevented the induction of this suppression, suggesting that a suppressor T cell-mediated phenomenon might be involved.

Immunoregulation in vivo is sometimes accomplished by the injection of monoclonal antibodies or immune sera. In attempting to apply this approach in the elucidation of the mechanism of γ-RCS induced suppression, we obtained an unexpected immunoregulatory effect with iv injected normal mouse serum (NMS), which had been intended to serve as a control. A further analysis of this phenomenon showed that absorption with normal spleen cells of any mouse strain removed the immunoregulatory factor from NMS, confirming its complete lack of strain specificity. As will be shown below we have further characterized this factor and found that it is non-dialyzable and regularly present in normal mouse and human serum, but not in plasma. This factor counteracts suppression when injected 1 day prior to antigen, frequently enhancing the response to SRBC above control levels, and is present in the supernatant of clotted mouse or human platelets as well as in human platelet lysates.

As shown in Table 1, injection of γ-irradiated RCS simultaneously with SRBC into SJL mice caused profound suppression of the PFC response in the spleen measured 4 days later, as described before. This effect was completely abolished by iv injection of 0.2 ml of a 1:4 dilution of NMS. The two experiments in Table 1 represent typical experiments, one in which the response was brought back to the control level by injection of NMS (Expt. 1) and one in which the NMS caused the response to become higher than in the control mice injected with SRBC alone (Expt. 2). Both syngeneic and allogeneic NMS caused the effect, and serum from 9-months-old had the same effect as serum from 2-months-old SJL mice. Absorption with unrelated (A/J mouse) spleen cells removed the activity from the NMS, while absorption with nu/nu (BALB/c) spleen cells did not.

The results in Table 2 confirm these findings and show that the same phenomenon is observed in BALB/c mice using BALB/c NMS. Again, absorption with BALB/c spleen removed the nonspecific enhancing activity, but absorption with nu/nu spleen cells did not. Although the degree of suppression obtained in BALB/c mice, either with γ-RCS (not shown) or with normal LN cells, was only moderate (~ 30°/°), both the suppression and the reversal to normal or above normal responses after NMS injection were statistically significant. Even serum obtained from a single mouse, clotted, spun, and reinjected into the same mouse caused the phenomenon of enhancement (Expt. 1, Table 2: autologous serum).

The results in Table 3 show that normal human serum (NHS) also had the immunoregulatory effect in the response of mice to SRBC. In two experiments (Expts. 1 and 2) the response was much higher than in controls not injected with serum or γ-RCS, but in Expt. 3 the response only went back to the control level, overcoming the γ-RCS induced suppression. Absorption of the NHS with mouse spleen or cells from a cytotoxic T cell line reduced or abolished the "contrasuppressive" effect of the NHS. The results in Table 3 suggest, in addition, that the effect is due to a non-dialyzable, heat-resistant factor.

It was of interest to determine whether NHS would also affect the rsponse to other antigens. We, therefore, performed an experiment in SJL mice with 10 μg TNP-Ficoll, to see the effect on a relatively thymus-independent response. The response was not much affected by injection of γ-RCS simultaneously with antigen, as shown previously, but it went from 43,650 ✕̸ 1.05 to 93,100 ✕̸ 1.1, when 0.05 ml NHS was injected iv on the day before antigen (Table 4).

It has been reported that human C5a (anaphylatoxin) may cause an enhancement of the immune response in mouse or in human cells in vitro and in mice in vivo [11]. Since clotting of blood can lead to activation of the C system, it seemed important to determine whether C5 deficient NMS could also cause the effect. Mice with haplotype k in the H-2S region are known to be deficient in C4 and mice of A strain background have low C5 levels. We, therefore, examined NMS from A.TL mice in A.TL and in A.TFR5 mice. A significant enhancement of the response was obtained in both these C4 and C5 deficient strains, no different from that observed in A.TFR3 and in SJL mice. It was, therefore, concluded that deficiency of these C factors did not much affect his phenomenon.

Another approach to the identification of the factor causing this effect was by use of proteolytic enzyme inhibitors. Addition of soybean trypsin inhibitor (10 μg/ml) or of tranexamic acid (50 μM) prior to clotting of blood resulted in NMS devoid of the effect in vivo (Fig. 1). Similarly 25μM amino-n-caproic acid (EACA) added to blood prior to clotting prevented the appearance of the factor in NMS, while addition of EACA to serum after clotting had no effect (Fig. 2). Plasma failed to affect the immune response in vivo, and, more significantly, clotting of plasma produced NMS which also lacked the effect (Figs. 1 and 2). The results in Fig. 1 show, in addition, that the effect of NMS can be seen in the absence of a suppressor cell inducing agent, since the mice in that experiment did not receive any γ-RCS injection.

In view of the lack of effect of NMS prepared from plasma in the absence of cells (Fig. 2) the results suggested that the factor was somehow generated from peripheral blood cells during clotting. Although interaction between B cells and plasminogen have been described [Maillard and Favreau, 1981], the first cells we turned our attention to were the platelets. The results in Fig. 2 show that the supernate from platelets, which had been spun down from lymphocyte and erythrocyte-depleted whole mouse blood and which were then allowed to clot by addition of thrombin (1 U per ml), significantly reversed suppression induced by γ-RCS (Fig. 3). The results in Table 6 demonstrate that similar results were obtained with releasate from human platelets incubated with thrombin. The releasate greatly enhanced the response to SRBC (Expt. 1), at least as much as was observed with serum.

TABLE 1

ANTIBODY PRODUCTION ENHANCING AND/OR SUPPRESSION REVERSING EFFECT OF NORMAL MOUSE SERUM IN SJL

Injected IV on			Expt. 1	Expt. 2
Day 0 SRBC (10^7)	Day 0 γ-RCS ($2x10^7$)	Day -1 NMS	PFC / Spleen	PFC / Spleen
+	-	-	11,700 ± 1.2	8,100 ± 1.1
+	+	-	2,300 ± 1.4 *o	1,100 ± 1.1
+	+	old SJL(unabs.)	11,500 ± 1.2 o	N.D.
+	+	young SJL(unabs.)	10,500 ± 1.1	
+	+	B10 X 9R(unabs.)	N.D.	28,200 ± 1.1
+	+	old SJL(abs. with A spleen)	4,700 ± 1.4*	N.D.
+	+	B10 X 9R(abs. with A spleen)	N.D.	1,100 ± 1.1
+	+	B10 x 9R(abs. with nude BALB/c spleen)	N.D.	34,700 ± 1.1

Results are expressed as geometric mean ± SE (n=5) of PFC per spleen assayed 4 days after IV injection of SRBC.

* N.S.

o $p<.01$.

TABLE 2

ANTIBODY PRODUCTION ENHANCING AND/OR SUPPRESSION REVERSING EFFECT OF NORMAL MOUSE SERUM IN BALB/c MICE#

INJECTED IV*			EXPT. 1	EXPT. 2
DAY 0 SRBC	DAY 0 SJL CELLS	DAY -1 BALB/c SERUM	PFC / SPLEEN	PFC / SPLEEN
+	-	-	15,800 ×÷ 1.1	21,900 ×÷ 1.0
+	+	-	10,700 ×÷ 1.1 O **	14,800 1.2+ ⊙
+	+	UNABSORBED (SYNGENEIC)	39,800 ×÷ 1.1 **	20,400 ×÷ 1.0 +
+	+	ABS. WITH BALB/c SPLEEN	11,500 ×÷ 1.1 O	15,100 ×÷ 1.2 ⊙
+	+	ABS. WITH NUDE BALB/c SPLEEN	40,700 ×÷ 1.1	31,600 ×÷ 1.1
+	+	UNABSORBED (AUTOLOGOUS)	44,700 ×÷ 1.1	N.D.

* NORMAL SJL LYMPH NODE CELLS (2×10^7) INJECTED IV TOGETHER WITH SRBC (5×10^6, EXPT. 1; 10^7, EXPT. 2).

** $P < .0001$.

O AND ⊙ N.S.

\+ $P < .002$.

\# RESULTS ARE EXPRESSED AS GEOMETRIC MEAN ×÷ SE (N=5) OF PFC PER SPLEEN ASSAYED 4 DAYS AFTER IV INJECTION OF SRBC.

TABLE 3

EFFECT OF HUMAN SERUM ON THE ANTI-SRBC RESPONSE OF SJL MICE #

Injected iv					
Day 0 SRBC (10^7)	Day 0 γ-RCS ($2x10^7$)	Day -1 Serum (50 µl)	Expt. 1 PFC/Spl	Expt. 2 PFC/Spl	Expt. 3 PFC/Spl
+	-	-	9,100 ×÷ 1.0	7,950 ×÷ 1.1	6,100 ×÷ 1.1
+	+	-	1,500 ×÷ 1.2	1,100 ×÷ 1.3	1,300 ×÷ 1.1
		Human (NHS)			
+	+	Fresh	26,900 ×÷ 1.1	17,400 ×÷ 1.1	7,250 ×÷ 1.2
+	+	Absorbed**	9,500 ×÷ 1.1	1,700 ×÷ 1.2	1,500 ×÷ 1.1
+	+	Heated(30 min. 56°C)	19,700 ×÷ 1.1	-	-
+	+	Dialyzed	-	-	5,900 ×÷ 1.1
		Mouse (NMS)			
+	+	Fresh	-	-	5,600 ×÷ 1.1

Results are expressed as geometric mean ×÷ SE (n=5) of PFC per spleen assayed 4 days after iv injection of SRBC.

** Absorbed at 4°C 1x (Expt. 1) or 2x (Expt. 2) with mouse spleen (10^8 cells/0.5 ml of 1:2 dil. NHS) or with CTLL-A11 (murine cytotoxic T cell line; Expt. 3: 3.5×10^7 cells/0.5 ml of 1:2 dil. NHS).

TABLE 4

EFFECT OF NORMAL MOUSE SERUM ON IMMUNE RESPONSE TO TNP-CONJUGATES IN SJL MICE

Injected i.v. on			Geom. Mean Anti-TNP PFC ± SE per Spleen in Response to
Day 0 Ag	Day 0 γ-RCS ($2x10^7$)	Day -1 (50μl)	TNP-Ficoll
+	-	-	43,652 ± 1.05
+	+	-	46,344 ± 1.05
+	+	Human Serum	93,110 ± 1.10

TABLE 5

PRESENCE OF ANTIBODY RESPONSE ENHANCING FACTOR IN C5 DEFICIENT MICE

Injected i.v. on			Geom. Mean. ×÷ SE of PFC/Spleen in Mice of Strain			
Day 0 SRBC (10^7)	Day 0 γ-RCS ($2x10^7$)	Day -1 A.TL NMS (50 µl)	A.TFR3 (fffsd)	A.TFR5 (ffkkd)	A.TL * (skkkd)	SJL (sssss)
+	-	-	7,250 ×÷ 1.1	6,900 ×÷ 1.1	6,150 ×÷ 1.2	3,000 ×÷ 1.1
+	+	-	1,950 ×÷ 1.1	3,250 ×÷ 1.1		730 ×÷ 1.6
+	+	+	30,200 ×÷ 1.1	22,900 ×÷ 1.1	19,500 ×÷ 1.2	9,950 ×÷ 1.5

* No γ-RCS injected in this experiment.
n = 4-5.

TABLE 6

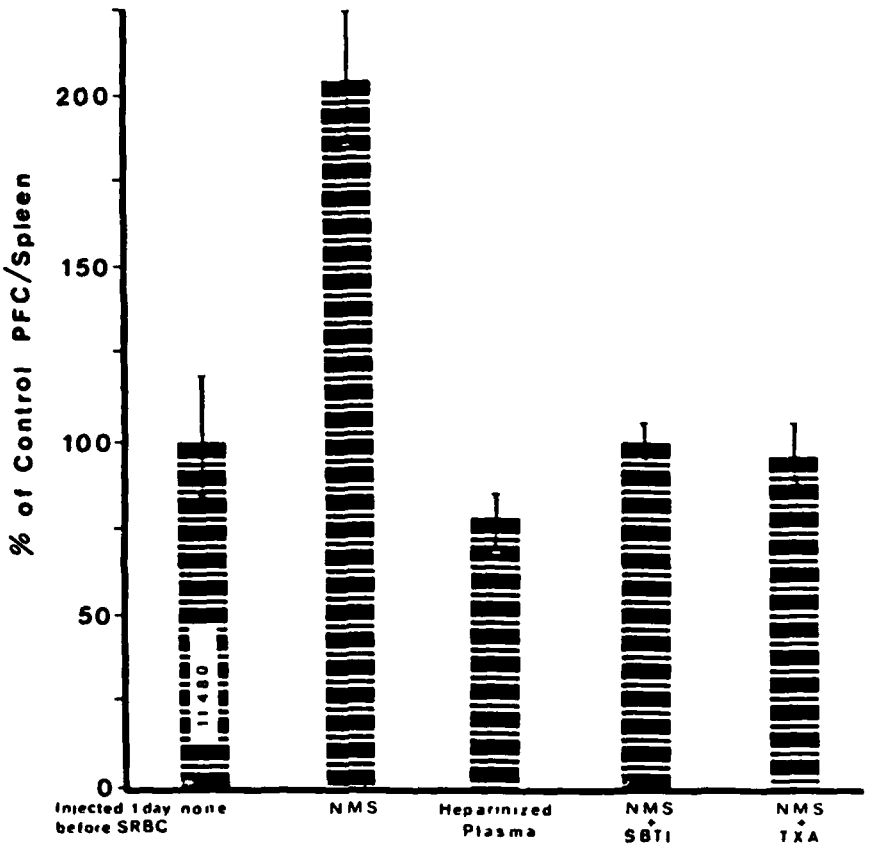
ENHANCEMENT BY HUMAN SERUM AND ISOLATED PLATELET RELEASATE OF ANTIBODY PRODUCTION IN SJL MICE**

Injected iv			Expt. 1	Expt. 2
Day 0 SRBC (10^7)	Day 0 γ-RCS ($2x10^7$)	Day -1 (50 μl)	PFC/Spl	PFC/Spl
+	-	-	9,100 ×÷ 1.0	7,950 ×÷ 1.1
+	+	-	1,500 ×÷ 1.2	1,100 ×÷ 1.3
+	+	NHS		
+	+	NHP		4,000 ×÷ 1.5
+	+	Platelet Lysate/Sup.	27,550 ×÷ 1.1	18,600 ×÷ 1.1
+	+	Thrombin#		3,600 ×÷ 1.3

* Human platelet releasate from thrombin incubated platelets (approx. 2×10^8/ml); 0.2 ml injected per mouse.

\# Amount of thrombin usedwas the same as that added to platelets to induce clumping (1 U/ml).

** Results are expressed as geometric mean ×÷ SE (n=5) of PFC per spleen assayed 4 days after iv injection of SRBC.



Legend for Figures:

Fig. 1. Effect of proteolytic enzyme inhibitors on appearance of antibody enhancing factor in serum during clotting of blood:

Splenic PFC response 4 days after iv injection of 10^7 SRBC (without γ-RCS) in SJL mice, who were also injected iv, 1 day before antigen, with 0.05 ml of normal mouse serum (NMS) which had been produced by clotting of whole blood in the presence of tranexamic acid (TXA, 50 μM) or of soybean trypsin inhibitor (SBTI, 10 μg/ml), or with plasma produced from heparinized blood. Results are expressed as °/• ($\bar{x}$ ± SE) of control (n = 5) and the total PFC per spleen in the control group is indicated in the bar.

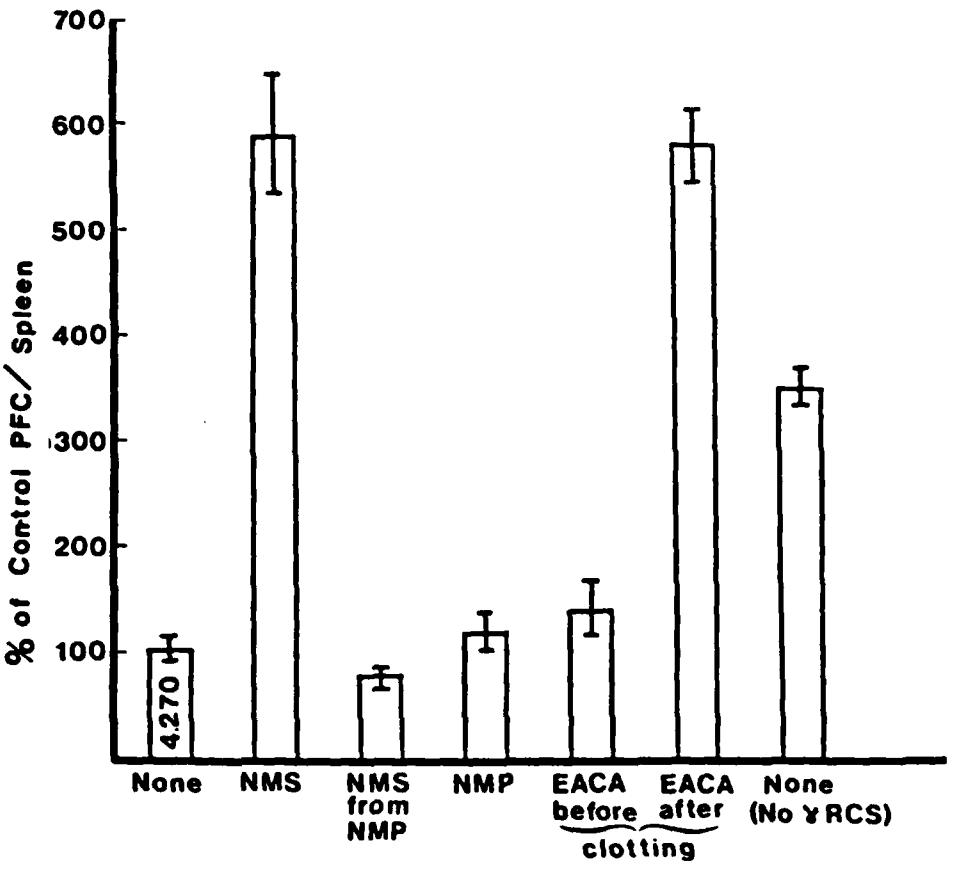


Fig. 2. Clotting in the presence of cells is needed for appearance of antibody enhancing factor in serum:

Splenic PFC response 4 days after iv injection of 10^7 SRBC and 2 x 10^7 γ-RCS in SJL mice, who were also injected iv, 1 day before antigen, with 0.05 ml of normal mouse serum (NMS), normal mouse plasma (NMP), NMS prepared after centrifugation of citrated blood by addition of Ca^{++} (NMS from NMP), or NMS to which EACA (25 μM) was added either before or after clotting. The response of mice injected with 10^7 SRBC without γ-RCS is also represented (None, no γ-RCS). Results are expressed as °/° ($\bar{x}$ ± SE) of control (n = 4-5) and the total PFC per spleen in the control group is indicated in the bar.

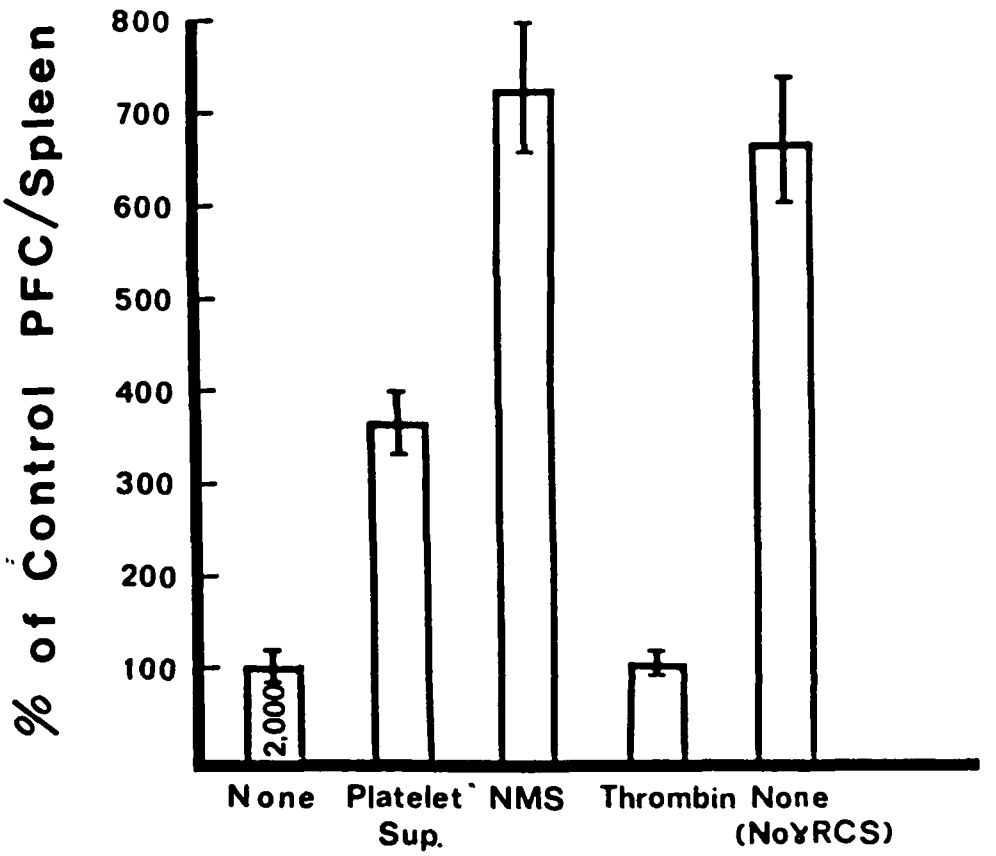


Fig. 3. Effect of platelet product on the immune response:

Splenic PFC response 4 days after iv injection of 10^7 SRBC and 2 x 10^7 γ-RCS in SJL mice, also injected iv, 1 day before antigen, with 0.05 ml of thrombin containing clotted platelet supernate, normal mouse serum (NMS) or thrombin alone. Results are expressed as °/• ($\bar{X}$ SE) of con trol (n = 5) and the total PFC per spleen in the control group is indicated in the bar.

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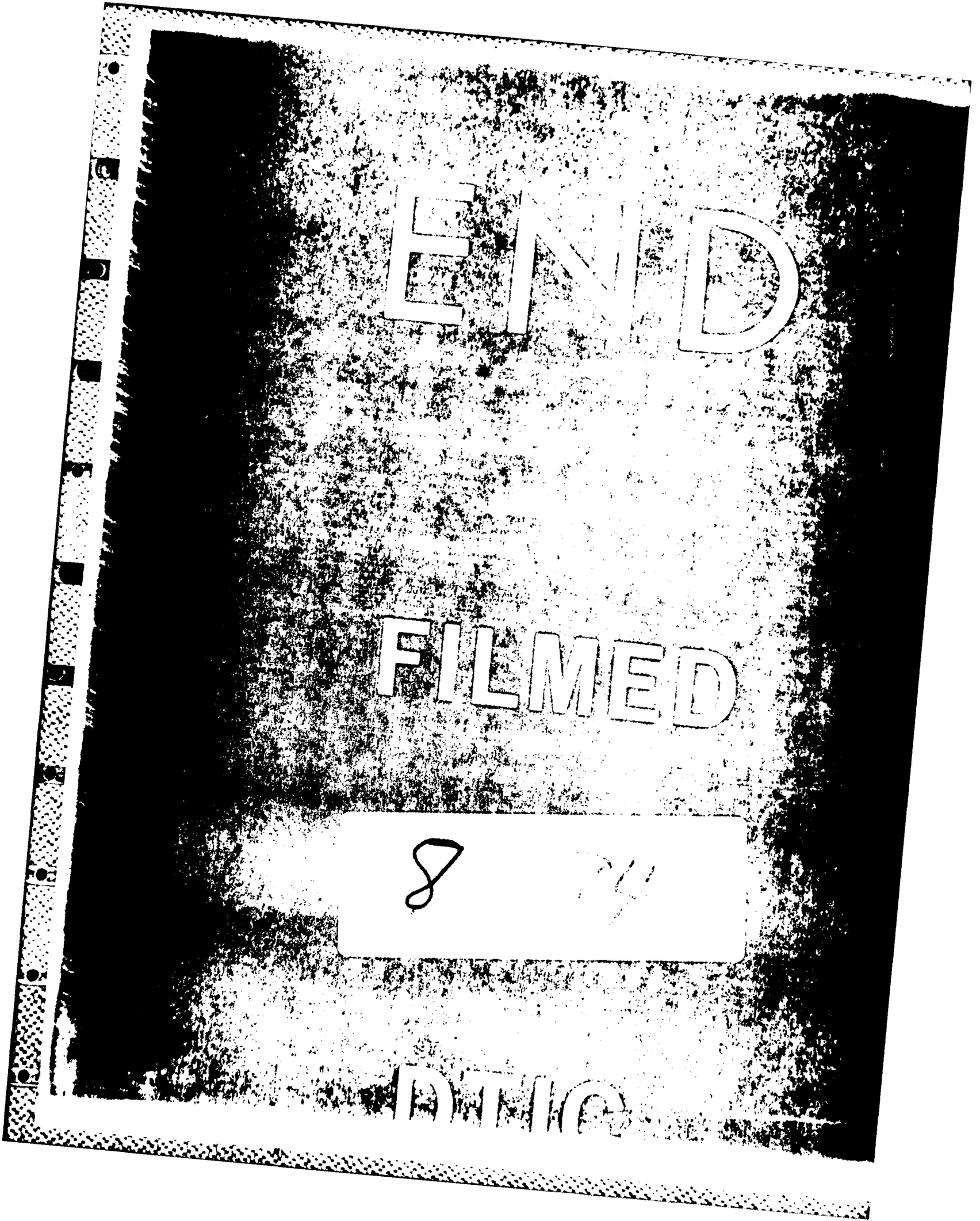
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