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Effect of Adjuvants on Response to Pneumococcal Polysaccharide Injected Intraperitoneally Platelet-Derived Immunoregulatory Activity.

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Studies on the immunoaugmenting effect of a platelet-derived serum factor were extended to include 1) identification of the factor as the α-granule constituent known as platelet factor 4. The cells with affinity for this factor were identified as Ly2$^+$, Ly2$^+$, Qa1$^+$, Qa4$^+$, Qa5$^+$, Ly6$^+$ and Ly1$^-$, L3T4$^-$, Thy1$^+$ cells. Effects of PF$_4$, detoxified endotoxin, IgD and 8-mercaptoguanosine on antibody production to pneumococcal polysaccharides types 3 and 14 were examined. Two aspects are being studied separately a) enhancement of the response (which is particularly good with endotoxin injected 4 days after antigen) and b) prevention of the tolerizing effect of slightly higher than optimal doses of polysaccharide (which may be possible with the platelet factor)

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I. Studies on the Immunoaugmenting Effect of a Platelet Factor

a) Identification of lymphoid cells capable of binding the factor

Summary of findings: It was established that both serum and platelet releasate (human or mouse) prepared either with thrombin or with calcium ionophore can prevent the suppression of the antibody (PFC) response to sheep erythrocytes (SRBC) induced either by syngeneic γ-irradiated lymphoma cells in SJL mice or by concanavalin A (con A) in all mouse strains tested. In many cases the response of mice injected with an appropriate amount of platelet releasate is above that of control, unsuppressed mice. Since it had been observed in previous experiments (the enclosed reprint) that splenic T cells could absorb the factor from serum, we attempted to characterize the cells in the spleen responsible for this binding of the factor. It was found that helper cells ($ly1^+$,$L3T4^+$) cells are not needed for this absorption, whereas $ly2^+$ cells are. The other cell surface markers found on the absorbing cells are Qa1,Ly22,Ly6,Qa4 and Qa5; suggesting that activated suppressor T cells are involved in removing the platelet factor.

Methods used in these experiments were described in the enclosed reprint or on footnotes to the tables. The antibodies used for killing of subpopulations in the presence of non-toxic rabbit C were: GK1.5 (rat IgG2b anti-L3T4); 19/178 (mouse IgG2a anti-Lyt2.2); SK70.94 (mouse IgG2a anti-Ly-m6.1E); T28.45.9 (mouse IgG2b anti-Ly-m 22.2); 2-2.1 (mouse IgG2b anti-Lyt1.2); B16-146 and B16-147 (mouse IgM anti-Qa4 and 5); and allo antiserum to Qa1.

The results can be seen in Tables 1 and 2 and in Figs 1A, B, and C. Table 1 shows examples of the reversal of conA-induced suppression by platelet releasate or serum in $CB6F_1$ mice. Although the effect of 50 μg con A was not completely reversed, that of 5 to 10 μg con A was and, in some cases, the control response to 2×10^7 SRBC alone (injected ip) was much lower than the response in mice receiving platelet factor and con A as well as antigen. In addition, the results of Expt 4 show that the serum could be injected before con A or 1 day after con A (just prior to antigen) with similar results.

The results in Table 2 show that spleen or lymph node cells absorb the suppression reversing effect from serum, while thymus cells do not. Two absorptions with 10^8 spleen cells per 0.5 ml of serum were sufficient to remove all activity, while two absorptions with 2×10^7 cells resulted in a greatly reduced activity.

The results in Fig 1A show that killing the $Lyt2^+$ (2.2^+) cells in spleen cells removes their ability to absorb, while removal of $GK1.5^+$ cells ($L3T4^+$) has no effect. The results in Figs 1B and C show that removal of the high $Lyt1^+$(1.2^+) bearing cells which can be killed by antibody +C has little effect on the ability of spleen cells to remove the activity while removal of $Qa1^+$, $Qa4^+$,$Qa5^+$,$ly6.1E^+$ or $Ly22^+$ cells in each case abolishes absorbing activity.

b) Identification of the immunoaugmenting platelet factor as platelet factor 4.

Summary of findings: When platelet suspensions are exposed to increasing concentrations of thrombin, the immunoaugmenting factor is released in

TABLE 1

ABROGATION OF CON A INDUCED SUPPRESSION OF THE ANTI-SRBC RESPONSE IN $CB6F_1$ MICE BY A PLATELET DERIVED SERUM FACTOR [a]

Day 0 SRBC [b]	Human Serum or Plt. Releasate	Day -1 Con A	Expt. 1 PFC/Spleen	Expt. 2 PFC/Spleen	Expt. 3 PFC/Spleen	Expt. 4 PFC/Spleen
+	None	-	99,200 (1.1)	79,400 (1.2)	5,600 (1.1)	8,700 (1.1)
+	None	50 µg	20,370 (1.1) [e]	-	-	-
+	Day -1 [d]	50 µg	65,300 (1.0) [e]	-	-	-
+	None	5 µg	-	84,760 (1.2) [g]	1,540 (1.3)	4,400 (1.1) [h]
+	None	10 µg	-	29,660 (1.2) [f]	-	-
+	Day -1 [c]	10 µg	-	63,250 (1.1) [f]	-	-
+	Day -1 [c]	5 µg	-	120,980 (1.1) [g]	77,600 (1.2)	-
+	Day -1 [d]	5 µg	-		44,900 (1.1)	51,590 (1.1) [h]
+	Day 0 [c]	5 µg	-	-	-	51,500 (1.1)

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

b) SRBC were injected ip: 1×10^8 in Expt. 1; 5×10^7 in Expt. 2; 2×10^7 in Expts. 3 and 4.

c) 50 µl serum/mouse, i.v.

d) 0.1 ml 1:50 diluted platelet releasate (10^9 platelets/ml) injected i.v. 1-2 hrs before Con A

p values are given for comparisons of values designated by same footnotes.

e) $p<0.0001$. f) $p<0.01$. g) $p<0.05$. h) $p<0.0001$

TABLE 2

ABSORPTION OF IMMUNOREGULATORY FACTOR FROM SERUM BY CELLS FROM DIFFERENT LYMPHOID ORGANS

Day 0 SRBC	Day 0* Human Serum	Day -1 Con A	Day 5** PFC/Spleen Geom. Mean ± S.E.
+	None	-	8,810 ± 1.1[a]
+	Unabsorbed	-	20,520 ± 1.2[a] [b]
+	None	+	2,930 ± 1.1
+	Unabsorbed	+	14,300 ± 1.1[b] [c] [d]
+	Absorbed 2×10^8 Spl	+	3,100 ± 1.1[c]
+	" 4×10^7 Spl	+	6,870 ± 1.1
+	" 4×10^7 LN	+	5,570 ± 1.1[d]
+	" $? \times 10^8$ Thy	+	13,870 ± 1.1
+	" 4×10^7 Thy	+	13,960 ± 1.1

* One ml (1:2 diluted) serum was absorbed twice for 30 min. at 4°C using half the number of $CB6F_1$ mouse cells indicated for each absorption.

Each $CB6F_1$ mouse received 0.2ml of 1:4 diluted unabsorbed or absorbed serum iv 1-2 hrs before 2×10^6 SRBC iv. Spl = spleen; LN = lymph node; Thy = thymus.

** p values are given for comparisons between values designated by the same footnote. n = 4.

a) p = .002.

b) N.S. (p=.081)

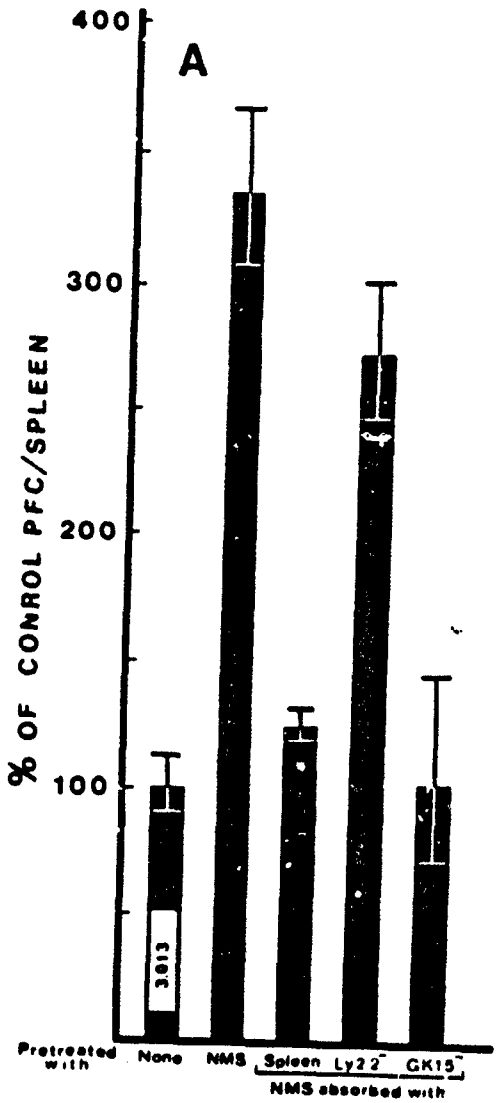
c) p<.0001.

d) p<.0001.

Fig. 1A, 1B, 1C

Identification of the subset and phenotype of cells able to absorb the immunoregulatory serum factor from serum.

Splenic PFC responses 5 days after simultaneous iv injection of 10^7 SRBC and 2 x 10^7 γ-RCS in SJL mice. Some groups of mice were also injected iv with 0.05 ml of unabsorbed normal mouse serum or serum absorbed (2X, see legend Table 3) with whole spleen cell suspension or with spleen cell suspension from which different subpopulations of cells were eliminated by treatment with mAbs. and complement (indicated as negative for the subpopulation which was killed). The response of mice injected with 10^7 SRBC without γ-RCS is represented as "None". Results are expressed as percent ($\bar{x}$ SE) of control (n=4) and the total PFC per spleen in control groups is indicated in the relevant bars for each of the three experiments. The responses are high in those groups of mice where the spleen cell suspension left after treatment with mAb and C was unable to absorb the augmenting factor from the serum.


A
400
300
200
100
% OF CONROL PFC/SPLEEN
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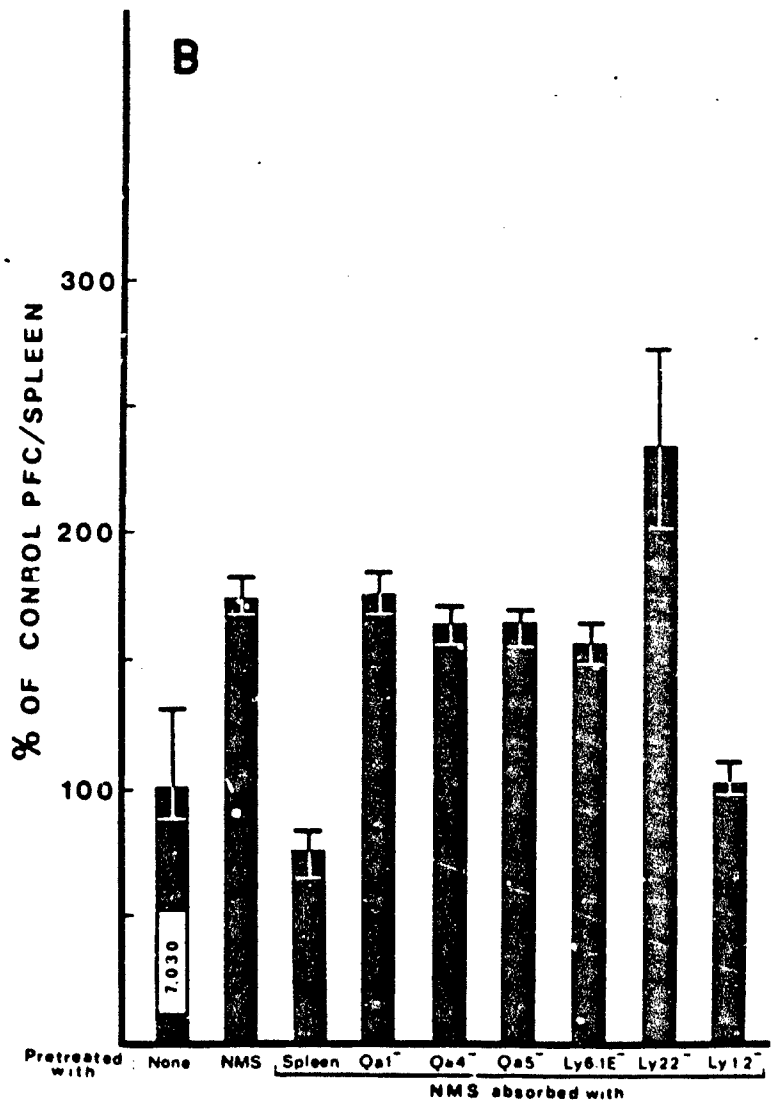
Pretreated with
None
NMS
Spleen Ly2.2⁻
GK1.5⁻
NMS absorbed with


B
% OF CONROL PFC/SPLEEN
300
200
100
7.030
Pretreated with
None
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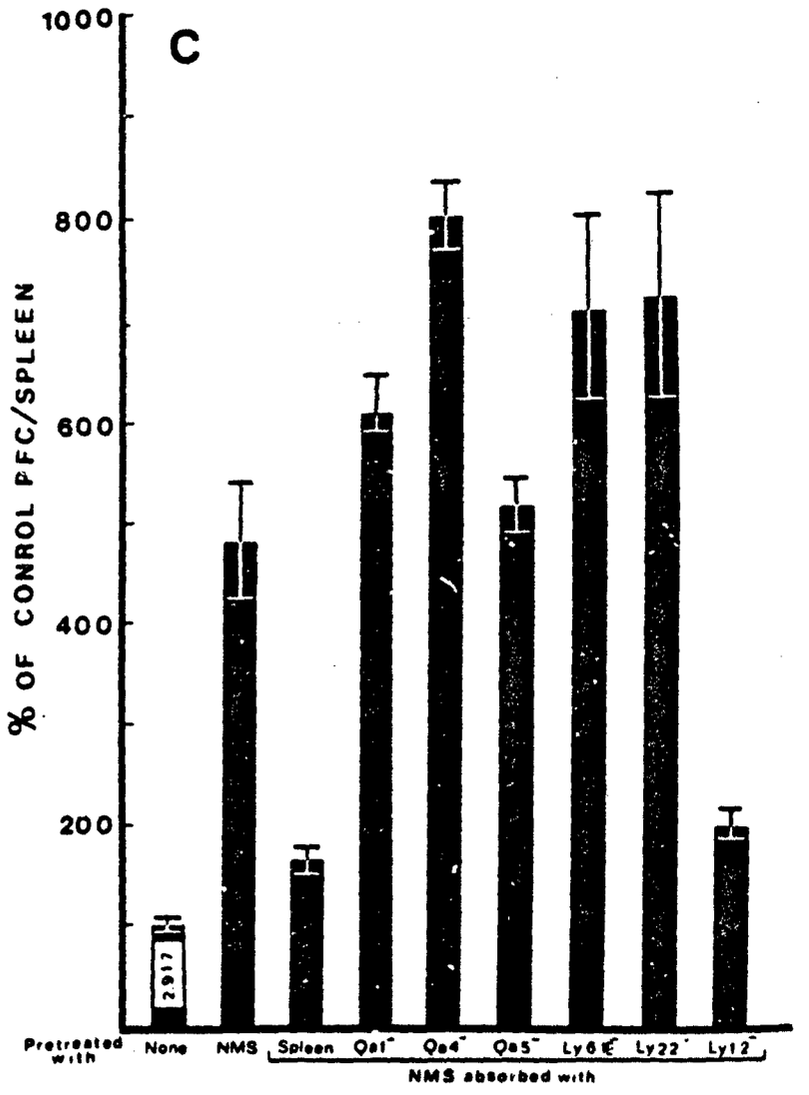
Spleen
Qa1⁻
Qa4⁻
Qa5⁻
Ly6.1E⁻
Ly22⁻
Ly12⁻
NMS absorbed with


C
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200
% OF CONROL PFC/SPLEEN
2.917
Pretreated with
None
NMS
Spleen
Qa1+
Qa4+
Qa5+
Ly6.1+
Ly22+
Ly12+
NMS absorbed with

parallel with α granule components. Heparin agarose absorbs the activity and purified platelet factor 4 (PF4) exhibits similar immunoregulatory activity as the whole platelet releasate. Other known constituents of α granules, such as platelet derived growth factor (PDGF) and low affinity (LA) PF_4 have little or no activity. Activity in human serum is neutralized by goat anti-human PF_4.

Methods used in these experiments were similar to those described in the enclosed reprint. Radioimmuno assay kits were used for assays of $LAPF_4$ and PF_4 (Amersham and Abbott Labs). Lactic dehydrogenase (LDH) and β-glucuronidase were assayed with kits from Sigma Chemical Co. Heparin-neutralizing activity was measured by the method of Harada and Zucker (Thromb. Diath. Haemorrh. 25,41,1984). Serotonin release was measured in platelets that had been incubated with 0.5 μM ^{14}-C-serotonin (Jerushalmy and Zucker, Thromb. Diath. Haemorrh. 15,413,1966) (56mCi/mMol, Amersham). Heparin agarose was purchased from Pierce Chem. Co.

Results are summarized in Tables 3-5, Figs. 2A and B, and Fig. 3.

Figs. 2A and B show dose-response curves of immunoregulatory activity obtained with serum (Fig. 2A) and with platelet releasate (Fig. 2B). The greatest effect was obtained with 0.2 ml of undiluted serum, but even 0.2 ml of 1:10 diluted serum increased the number of PFC above the control value. With releasate the greatest effect was noted after injection of material released from about 3 X 10^6 platelets, and releasate from 10^6 platelets are still effective.

Fig. 3 shows the simultaneous release of immunoregulatory activity and the known α-granule constituents: PF4, serotonin and β-glucuronidase by different concentrations of thrombin added to a human platelet suspension and incubated for 60 minutes. LDH was measured as an indication of platelet lysis, since it is not present in α granules and should not be released by thrombin. Its concentration in the releasates was less than 8% of its concentration in the platelets (as measured after completely lysing the platelets).

Tables 3-5 show the data which suggest that the immunoregulatory activity is due to PF4. The purified PF4 and LAPF4 preparations tested (Table 4) were gifts from Dr. S. Niewiarowski (Temple Univ., Philadelphia) and were isolated according to Varma et al (Biochim. Biophys. Acta 701,7,1982). Both the binding to heparin and the neutralization of the activity by anti-PF4 support the finding that isolated PF4 has the activity.

The effect of 0.2-0.6μg, PF4 was as great as that of the releasate from $2X10^7$ platelets (Table 4), which is in agreement with the observation that platelets contain .18μg of PF4 per 10^7 platelets (Files et al., Blood 58, 607, 1981). The efficiency of such small amounts of this immunoaugmenting protein is remarkable and also agrees with the effectiveness of 50μl of serum, since human serum contains approximately 5μg PF4 per ml (Lonky and Wohl, J. Clin. Invest. 67, 817, 1981).

II <u>Studies on the immune response of mice to pneumococcal polysaccharides types 3 and 14</u>.

a) <u>Effect of antigen dose and mouse strain</u>. It is known that BALB/c mice are high responders to type 3 polysaccharide and in our previous report we showed

TABLE 3

ABSORPTION OF ACTIVITY BY HEPARIN-AGAROSE

Mice Injected With[a]		Geom. Mean PFC/Spleen on Day 5		
5 µg Con A	Additional Material	Expt. 1	Expt. 2	Expt. 3
-	None	9,330	15,850	7,700
+	None	4,020*	8,510	1,760
+	Rel.	11,450*	22,910	ND
+	Rel. absorbed with heparin-agarose	3,000*	7,410	4,210
+	Heparin-agarose eluate from Rel.	10,540*	26,060	11,940
+	Rel. absorbed with agarose	ND	ND	10,580

[a] Mice injected iv on day -1 with 5 µg Con A and on day 0 with 2 x 10^6 SRBC and platelet releasate (Rel.) or derivatives.

*Antilog of standard error = 1.2; for all other determinations, it is $\leq$ 1.1. (n=4).

TABLE 4

IMMUNOREGULATORY ACTIVITY OF PLATELET α GRANULE CONSTITUENTS

Mice Injected With*		Geom. Mean PFC/Spleen+ on Day 5		
5 μg Con A	Additional Material	Expt. 1	Expt. 2	Expt. 3
-	None	16,090	12,450	32,590
+	None	8,000	4,880	12,920
+	Rel.	14,190	29,920	30,740
+	PF4*	ND	22,910	34,420
+	LA-PF4**	ND	9,440	ND
+	PDGF≠	8,370	ND	ND

* Mice injected iv on day -1 with 5 μg Con A and on day 0 with 2 x 10^6 SRBC plus releasate (Rel.) from 2 x 10^7 platelets or purified platelet proteins.

** 0.6 μg (Expt. 2) or 0.2 μg (Expt. 3) per mouse.

≠ 0.003 μg per mouse.

+ S.E. (antilog) $\leq$ 1.1; n = 4.

TABLE 5

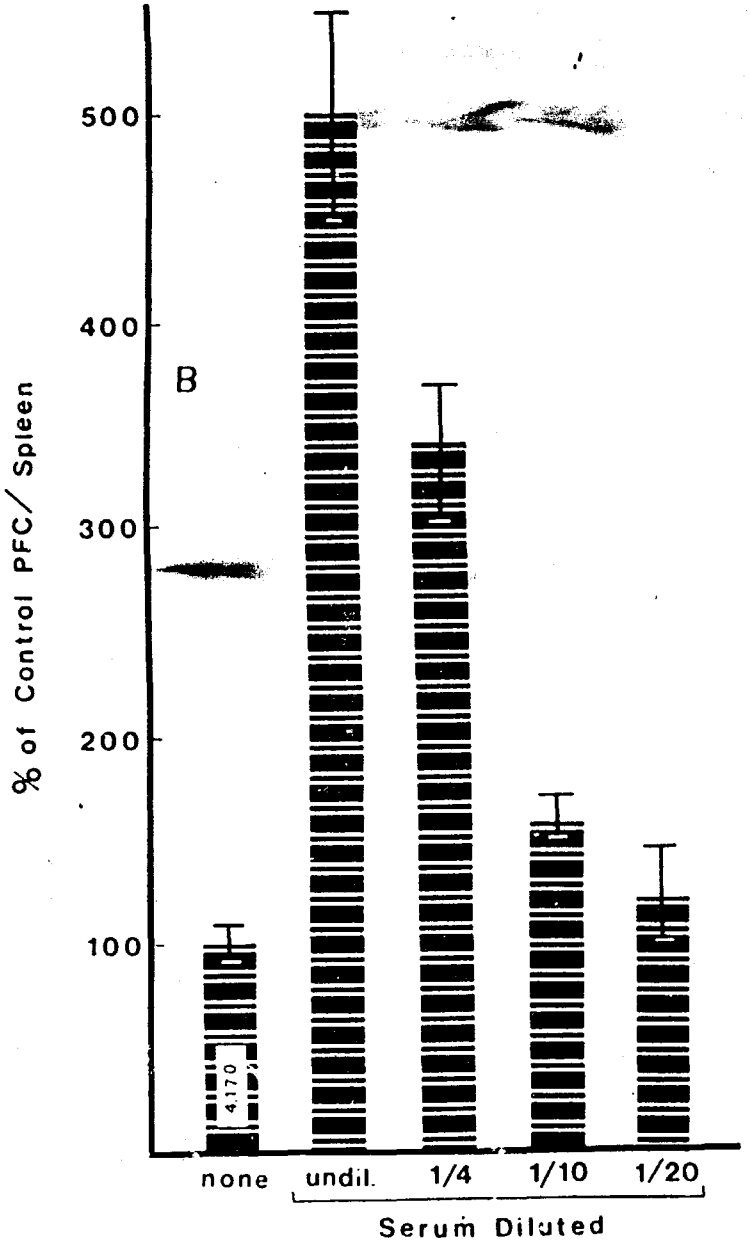
REVERSAL OF IMMUNOREGULATORY ACTIVITY IN HUMAN SERUM BY GOAT ANTISERUM TO PF-4

Mice Injected With*		Geom. Mean PFC/Spleen** on Day 5
5 μg Con A	Additional Material#	
-	None	8,720
+	None	4,380
+	Human Serum	51,590
+	Human Serum + goat anti-PF4	13,190
+	Human Serum + Nl goat serum	49,470
+	Goat anti-PF4	10,000

** S.E. (antilog) $\leq$1.1; n = 4.

* Mice injected with Con A and SRBC as in Table 4.

Human serum (0.05 ml) and/or goat antiserum to PF4 (0.008 ml) or normal goat serum (0.008 ml) were incubated for 20 min. at 4°C prior to injection.



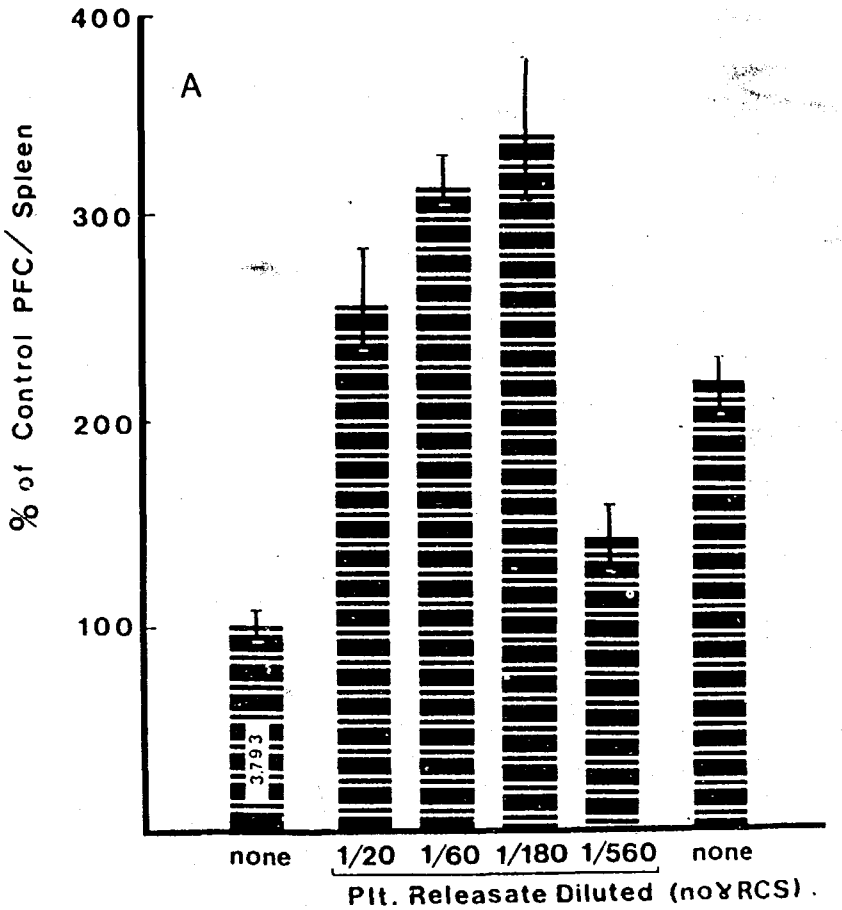


Figure 2 -- Splenic PFC produced in response to injection of suppressive agent (γ-RCS), SRBC, and different concentrations of (A) human serum and (B) releasate from 10^9 platelets per ml. The geometric mean for PFC per spleen in the control suppressed mice is indicated in the first bars.

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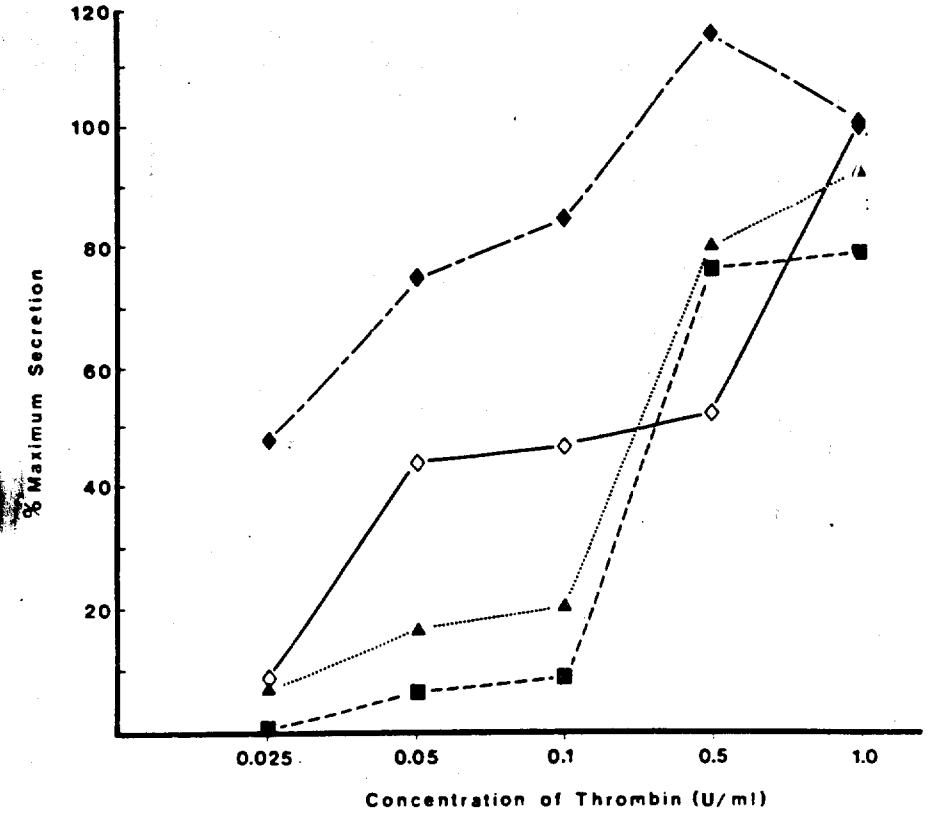

Figure 3 -- Secretion of PF4 (◆), IA (◇) ^{14}C-serotonin (▲), and β-glucuronidase (■) from platelets stimulated with thrombin. The geometric mean for PFC per spleen was 4126 in mice injected with suppressive agent (γ-RCS), SRBC and releasate made with 1 U/ml thrombin, and was 339 in mice given γ-RCS and SRBC and no releasate.

that BALB/c mice also respond well to type 14. The optimal antigen dose for the two polysaccharides for induction of a primary response differs markedly, however. While for type 3, 0.1 μg is better than 1μg and much more immunogenic than 5 or 10 μg in all strains tested (BALB.B, CB6 F_1 and BALB/c), the optimal dose for type 14 is much higher:25 μg is better than 0.5 μg or 5 μg in BALB/c mice, as well as in outbred mice. In $CB6F_1$ mice the primary response, even after 25 μg intraperitoneally, is low. Similarly, the optimal dose for killed bacteria is lower for type 3 than for type 14 pneumococci, i.e. 10^8 for type 3 and 5×10^8 for type 14 (data not shown).

b) Effect of exposure to pneumococcal polysaccharide on the ability of mice to respond to killed penumococcal bacteria (Tables 6 and 7).

Although 7S antibody is generally not detected in sera from mice taken 5 days after a primary injection of polysaccharide and is low, if detected, after injection of killed bacteria, a challenge with killed pneumococci 10-14 days after priming with polysaccharide frequently brings forth a slightly higher 7S response. However, this response is not sustained and the antibody response tends to be lower after priming with high than after priming with low doses of polysaccharide. This is particularly true with type 3 polysaccharide, which in all strains tested induces tolerance when injected in doses of 5-10 μg.

Priming with whole bacteria is somewhat more effective than with polysaccharide, but too high a dose of killed pneumococci particularly in the case of type 3, also induces tolerance. It may, therefore, be very important to attempt to overcome this tendency to induce tolerance at the time of primary immunization, since an adjuvant effect simply on the magnitude of the response does not necessarily counteract tolerance (suppressor T cell) induction.

In previous experiments we have shown that injection of IgD (0.5 ml ascites fluid containing approx. 1.5 mg TEPC-1017 IgD myeloma protein) together with the primary injection of a thymus dependent antigen, such as sheep erythrocytes or TNP-hemocyanin, causes a marked enhancement of the 7S response, to a second injection of the same antigen given 2 weeks after the primary. We, therefore, examined the effect of injection of IgD together with the primary (tolerizing) injection of polysaccharide. There was no detectable effect on the primary or secondary responses to either type 14 or type 3 pneumococci (Tables 6 and 7).

In our previous progress report we showed that the response to a conjugate of type 3 polysaccharide with MTP is somewhat higher than that to a similar dose of the uncongugated polysaccharide. However, injecting this conjugate for the primary injection did not cause an increase in the secondary response (Table 7). It should be noted, that both with type 14 and with type 3 polysaccharide the survival upon challenge with viable pneumococci was perhaps slightly improved by some of these pretreatment regimens (Expt. 1, Table 6 and Expt. 2, Table 7). In other experiments, however, pretreatment with polysaccharide alone followed by challenge with killed bacteria was sufficient to induce some protective immunity (Expt. 2, Table 6 and Expt. 3, Table 7).

Preliminary experiments (not shown here) suggest that pretreatment with IgD containing ascites fluid may enhance primary responses to type 14

TABLE 6

RESPONSE TO $5x10^8$ KILLED PNEUMOCOCCI (TYPE 14) AFTER PRIMING WITH VARIOUS DOSES OF PNEUMOCOCCAL POLYSACCHARIDE (PS)

Strain	Primary Injection (Day 0)	Secondary* response (Log_2 1/titer±SE) on Day 19 19S	on Day 19 7S	on Day 24 or 29 19S	on Day 24 or 29 7S	Survival on Viable Bact Challenge %
BALB/c	0.001 µg ps	9.5±0.2	6.6±0.1	8.3±0.1	7.6±1	0
	25µg ps	10.1±0.4	5.9±0.2	8.3±0.3	7.2±0.3	0
	25 µg ps + IgD+	10.1±0.6	6.6±0.5	8.9±0.4	7.0±0.6	40
	25 µg ps+LPS**	9.9±0.3	6.3±0.4	8.2±0.4	7.1±0.4	20
	None	8.5±0.9	4.8±0.8	7.0±0.7	6.0±1.0	0
CB6 F_1	1µg ps	8.2±0.5	5.8±0.5	6.5±0	<3	67
	25 µg ps	9.2±0.4	3.5±0.8	5.7±0.5	<3	67
	$5X10^8$ killed bact.	10.8±0.5	6.4±0.3	7.8±0.5	4.4±0.5	67
	None	7.4±0.4	3.1±0.5	4.8±0.1	<3	0

*Mice received an ip injection of killed type 14 pneumococci 14 days after priming and were bled 5 and 10 days (BALB/c) or 5 and 15 days ($CB6F_1$) later (n=3-6).

+IgD=0.5 ml ascites fluid from TEPC 1017 bearing mice, ip, containing approximately 1.5 mg IgD.

**Two days after priming mice received LPS (E.coli) 10 µg, ip. Response was similar to that obtained with detoxified endotoxin (not shown).

TABLE 7

TOLERIZING EFFECT OF TYPE 3 POLYSACCHARIDE (ps) ON SUBSEQUENT RESPONSES TO KILLED PNEUMOCOCCI INJECTED 14 DAYS LATER

Strain	Primary Injection	Secondary* response (Log_2 1/titer±SE) on Day 19	Survival on Viable Bact Challenge %
		19?	
BALB/c	0.1 µg ps	7.5±0.2	ND
	1µg ps	6.7±0.3	ND
	5µg ps	<3	ND
	None	7.5±0.6	ND
BALB.B	1µg-MTP ps	3.7±0.7	20
	1ug ps	5.5±0.3	0
	1µg ps + IgD[a]	3.0±0.5	20
	10µg ps	<3	0
	10µg ps + IgD[a]	<3	0
	None	3.6±0.6	0
$CB6F_1$	0.1 µg ps	6.8±0.1	33
	1µg ps	5.7±0.1	33
	10^8 killed bact	6.5±0.5	67
	10^9 killed bact	<3	33
	None	5.8±0.4	0

*Injection of killed penumococci type 3 on day 14:10^8 ip in BALB/c and $CB6F_1$, 5 x 10^8 ip in BALB.B mice (n=3-5). All 7S titers were <3.

[a]IgD=0.5 ml TEPC-1017 ascites ip on day 0.

polysaccharide, as was previously shown to be the case with a variety of antigens (Coico et al., Nature 316:744, 1985; Xue, et al., J. Exp. Med. 159:103, 1984). However, there is some variability in the magnitude of this effect, the reason for which needs to be further examined and may be related to the observation that IgD cannot prevent the induction of tolerance.

c) Effect of platelet releasate and normal mouse serum on responses to pneumococcal polysaccharides (Table 8).

Simultaneous injection of platelet releasate or serum with type 14 pneumococcal polysaccharide did not significantly enhance the primary response. However, the responses of such mice reinjected with killed pneumococci 2 weeks later was higher than in mice receiving polysaccharide alone for the primary injection. This effect was seen on both the 19S and the 7S responses, but more experiments are needed to study the isotype distribution of this enhanced response and to determine whether the tolerance obtained with type 3 pneumococcal polysaccharide can be reversed by injection of the platelet factor.

d) Effect of detoxified endotoxin (D-LPS) on the response to pneumococcal polysaccharide (Table 9).

It was surprising to find that D-LPS (Ribi Immunochem. Res., Inc.) did not much affect the primary response to type 14 polysaccharide when injected simultaneously with the polysaccharide (data not shown). Attempts were made, therefore, to enhance the response by injecting the D-LPS on various days after the polysaccharide. It was found that injection on day 4 had a particularly stimulating effect on both 19S and 7S antibody production in the primary response. The secondary response to killed bacteria in such D-LPS treated mice was also much higher than in control mice, again for both 19S and 7S.

e) Effect of 8-mercaptoguanosine on the response to pneumococcal polysaccharide (Table 10).

Injection of 30 mg 8-mercaptoguanosine together with 25 µg type 14 polysaccharide did not much enhance the response and in some cases even caused an inhibition, perhaps because it tended to make our BALB/c mice sick (not shown). However, when this drug was injected in doses of 10 mg per day on days 1,2, and 3 after antigen, it caused a moderate enhancement of the 7S antibody response, particularly when given again after a second injection of polysaccharide. There was no impressive degree of protective immunity in these mice, but it was usually higher than in mice injected with polysaccharide alone.

In Summary: The most promising method to enhance primary responses to type 14 polysaccharide in these experiments appears to be through injection of detoxified LPS 4 days after a primary injection. This enhancing effect carries over into the secondary response, but the effect on the secondary response is not better than that of platelet factor. Both of these agents still need to be examined for their effects on the response to type 3 polysaccharide.

TABLE 8

THE EFFECT OF PLATELET FACTOR ON THE IMMUNE RESPONSE OF BALB/c MICE TO TYPE 14 PNEUMOCOCCAL POLYSACCHARIDE

Primary Injection of 25 µg pn.ps. and:	Response	Log_2 1/serum agglutinin titers ±SE* on Day 5 (19) 19S	Day 5 (19) 7S	Day 10 (24) 19S	Day 10 (24) 7S	Survival On Viable Bact. Challenge %
None	Primary	7.0±0.2	ND+	4.6±0.3	3.1±0.3	ND
Platelet Releasate[α] (Day 0)		6.8±0.3	ND	4.9±0.4	3.3±0.3	ND
Platelet Releasate (Days 0, 7)		6.9±0.1	ND	5.1±0.2	3.2±0.3	ND
NMS (Day 0)**		5.4±0.3	ND	3.7±0.7	2.4±0.2	ND
NMS (Days 0,7)		6.9±0.5	ND	5.5±0.5	3.6±0.5	ND
None	Secondary	7.1±0.8	2.5±0.3	6.2±0.4	4.3±0.5	40
Platelet Releasate (Day 0)		9.9±0.1	3.5±0.2	8.1±0.3	5.9±0.4	100
Platelet Releasate (Days 0,7.14)		9.9±0.3	3.7±0.2	7.2±0.1	5.3±0.4	80
NMS (Day 0)		9.0±0.2	2.9±0.4	6.8±0.1	4.6±0.1	100
NMS (Days 0, 7, 14)		9.3±0.4	4.4±0.5	7.9±0.2	6.0±0.6	100

*Mean Log_2 ± SE (n=5) are given for reciprocals of titers of sera taken 5 and 10 days after primary ip injection of pn.ps. or after the secondary ip. injection of killed bact. 2 weeks later.
+ND=not done

**NMS=normal mouse serum, 0.05 ml iv

[α]Platelet Releasate from 2 x 10^7 human platelets was injected iv.

TABLE 9

EFFECT OF DETOXIFIED ENDOTOXIN (S. TYPHIMURIUM) ON THE IMMUNE RESPONSE OF BALB/c MICE TO TYPE 14 PNEUMOCOCCAL POLYSACCHARIDE

10 µg LPS[a] ip injected on	Response	Log_2 1/serum agglutinin titer ±SE* on Day 5 (19) 19S	Day 5 (19) 7S	Day 10 (24) 19S	Day 10 (24) 7S	Survival On Viable Bact. Challenge %
None	Primary	6.0±0.8	ND[+]	4.5±0.6	3.0±0.8	ND
Day 2		6.9±0.4	ND	5.8±0.4	4.3±0.7	ND
Day 3		6.0±1.1	ND	5.1±0.7	3.3±0.6	ND
Day 4		8.6±0.4	ND	7.3±0.6	6.0±0.9	ND
Day 5		6.3±1.1	ND	5.3±0.8	4.5±0.6	ND
None	Secondary	7.1±0.4	3.9±0.1	7.1±0.3	5.4±0.9	25
Day 2		8.3±0.2	4.2±0.4	8.1±0.3	6.3±0.4	25
Day 3		8.3±0.2	4.2±0.2	9.2±0.4	6.4±0.4	25
Day 4		9.3±0.3	5.6±0.4	9.8±0.1	8.4±0.3	25
Day 5		9.5±0.4	4.8±0.1	8.8±0.	7 1±0.2	25

*Mean Log_2 ± SE (n=4) are given for reciprocals of titers in sera taken 5 and 10 after primary injection of 25 µg pn. ps. type 14 ip, or in sera taken 5 (Day 19) and 10 days (Day 24) after the secondary injection of $5x10^8$ killed pneumococci given on day 14.

[+]ND= not done

[a]Purchased from Ribi Immunochem. Res., Inc.

TABLE 10

THE EFFECT OF 8-MERCAPTOGUANOSINE (8-MG) ON THE RESPONSE TO TYPE 14 PNEUMOCOCCAL POLYSACCHARIDE (pn.ps.)

Primary Injection with 25 µg pn. ps and:	Response	Log_2 1/serum Agglutinin Titers on* Day 7 (21) 19S	Day 7 (21) 7S	Day 14 (28) 19S	Day 14 (28) 7S	Survival On Viable Bact. Challenge °/o
None	Primary	4.2±0.5	3.7±0.5	3.7±0.5	2.2±0.8	ND+
8-MG[α]		5.2±1.0	4.4±0.7	3.4±0.6	3.3±0.4	ND
None	Secondary	6.7±0.4	2.3±0.3	7.3±0.3	2.6±0.3	0
8-MG[α]		7.0±0.3	4.0±0.7	8.6±0.5	5.7±0.6	20

*Mean Log_2 ±SE (n=5) are given for reciprocals of titers of sera raken 7 and 14 days ıfter primary or secondary ip injections of 25 µg pn. ps. with a 14 day interval.

+ND=not done

[α]8-mercapto guanosine dose was 10Mg per injection, ip, given on days 1,2 and 3 of the primary and on days 15,16,17 during the secondary response.