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Rheumatoid factors in mice: non-specific activators of heterophile rheumatoid factor production

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Summary. Although possibly the best, LPS is by no means the only non-specific activator of immune responsiveness (AIr). Plant lectins such as PHA and PWM have some ability to stimulate both total IgM production (reverse IgM PFC) and more particularly heterophile and homophile IgM rheumatoid factor (RF) responses, together with IgM 'antibody' responses to target antigens such as xenogeneic red blood cells or haptens such as TNP. Some parasitic infections and/or parasite antigens were shown to be powerful non-specific AIr, while the injection of dextran sulphate was shown to lead to a great increase in spleen cellularity and total IgM (reverse) PFC but failed to stimulate a heterophile (BIG) RF PFC response.

INTRODUCTION

Non-specific activation of immune responsiveness is not the sole prerogative of lipopolysaccharides (LPS) of Gram-negative bacteria. Phytohaemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM) are among plant lectins which have been described as being activators of immune responsiveness (AIr) (Andersson, Sjöberg & Möller, 1972; Parkhouse, Janossy & Greaves, 1972). Dextran sulphate and a purified protein derivative of tuberculin (PPD) (Nilsson, Sultzer & Bullock, 1973) have also been shown to have a similar stimulatory activity. Most of the antibody measured in the experiments described in the publications just mentioned was directly against experimental antigens such as xenogeneic red blood cells (sheep, horse) phenyl-based haptens (TNP-, DNP-, NNP-, NIP-) or more recently against DNA (Izui, Zaldivar, Scher & Lambert, 1977a; Izui, Kobayakawa, Zyrd, Louis, & Lambert, 1977b) and cryptic antigenic determinants revealed by bromelain or neuraminidase treatment of syngeneic RBC (Steele & Cunningham, 1978). Both rodent malarial infection (Freeman & Parish, 1978) and experimental infection with African trypanosomiasis (Kobayakawa, Louis, Izui & Lambert, 1979) have been shown to activate non-specifically aspects of the immune response. Recently LPS has been shown to stimulate the production of both homophile and heterophile IgM anti-IgG rheumatoid factors (Dresser & Popham, 1976; Dresser, 1978; Izui, Eisenberg & Dixon, 1979).

In this paper, experiments are reported in which a number of substances have been tested together with some parasitic infections, in CBA mice, for their effectiveness as non-specific AIr, and in particular for the stimulation of heterophile rheumatoid factor (RF)-producing cells. This activity is detected as indirect (anti-μ developed) plaque-forming cells (PFC) which produce a product with a low avidity antibody-like specificity for bovine IgG (BIG).

MATERIALS AND METHODS

CBA male mice aged 6-12 months were used in the experiments described in this paper. The methods used are given in a previous paper (Dresser & Popham,

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Table 1. Activation of a heterophile RF (BIG) response by different lipopolysaccharides

Activator	Dose (μg)	PFC/spleen*
None	—	0
Con A	25	0
LPS† 0901 B	25	7,725
LPS 0901 W	25	8,650
LPS 0127 W	25	11,600
LPS *Serratia marcesens* B	25	15,500
LPS 055 B5 B	25	7,850
Lentinan‡	250	1,250

* Anti-BIG, μ-developed, day 3 (pool of 4 spleens for each group).
† All LPS purchased from Difco.
‡ Gift from Dr J. Hamuro, prepared from *Lentinus edodes* (Berk.) Sing. (see Maeda, Hamuro, Yamada, Ishimura & Chihara, 1973). Lentinan is a T-cell adjuvant.

1980) in which modifications to a standard procedure for the Jerne haemolytic plaque assay (Dresser, 1978a) are described. Details of substances tested for the ability to act as AIr are given in the footnote to the tables or the legends to the figures. With the exception of a suspension of *Corynebacterium parvum* all substances being tested for activity as AIr were injected intraperitoneally (i.p.). Unless stated otherwise,'LPS'-refers to lipopolysaccharide from *Salmonella typhosa* 0901 (Boivin preparation) (Difco).

RESULTS

It has been suggested that Boivin (B) preparations of lipopolysaccharides owe their *in vitro* mitogenicity for the lymphoid cells of C_3H/HeJ mice, to the presence of lipoprotein (Sultzer & Goodman, 1976; Morrison, Betz & Jacobs, 1976): the C_3H/HeJ strain of mice is a non-responder (*in vitro* mitogenicity) to Westphal (W) phenol-extracted LPS (Watson & Riblet, 1974). In Tables 1 and 6 it can be seen that contrary to expectations based on previous work cited above, LPS W preparations are slightly better than LPS B preparations as *in vivo* AIr. Table 2 shows that the injec-

Table 2. Different patterns of responsiveness elicited by various non-specific activators of the immune response

Activator	Total IgM PFC/spleen (±SE)	Responses as % of total IgM	
		BIG (RF)	SRBC (direct)
Nil	54,063 (44,189)	0·12	0·04
20 μg LPS	6,081,250 (515,931)	2·47	0·13
20 μg lipoprotein† *Salm. salm.*‡	1,583,750 (99,945)	10·12	0·12
20 μg lipoprotein *Salm. poona.*	745,625 (109,223)	2·46	0·16
100 μg MDP§	175,000 (42,365)	0·07	0·24

* Response measured 4 days after i.p. injection of indicated materials into groups of four mice.
† Lipoprotein preparations were a gift from Dr D. A. L. Davies.
‡ *Salmonella salmonitis* grown on a beef broth medium.
§ Muramyl dipeptide (peptidoglycan) a gift from Dr E. Lederer.

Table 3. Dextran sulphate can activate some immune responsiveness but fails to increase the level of heterophile RF or anti-TNP PFC

Activator	PFC/spleen*			
	Total IgM	BIG (μ dev.)	SRBC (direct)	TNP_{Hi} (direct)§
None	37,750	0	25	21,475
LPS (20 μg)	6,281,200	394,200	7200	460,800
DS†(100 μg) Serva	272,425	0	475	22,775
DS (100 μg) Pharmacia	229,225	0	775	17,725
PVP‡ (50 μg)	157,275	325	425	28,575

* Day 4, CBA males, corrected for PFC against uncoated SRBC.
† Dextran sulphate.
‡ Polyvinyl pyrolidone (BDH).
§ TNP-coupled to SRBC at a concentration of 40 mg/ml packed RBC by the method of Rittenberg & Pratt (1969).

Table 4. A comparison of three 'mitogenic' substances as non-specific AIr

AIr (dose)	Cells/spleen $\times 10^6$ (±SE)	Total IgM PFC/Spleen (±SE)	Response as % total IgM (±SE)			
			MIG (RF)	BIG (RF)	TNP-$_{Hi}$	SRBC
Nil (control)	85·8 (6·7)	521,250 (44,890)	70·1 (9·3)	0·12 (0·05)	1.18 (0·25)	0·05 (0·02)
LPS 20 μg	131·8 (12·5)	7,700,000 (491,172)	75·7 (10·1)	3·23 (0·58)	0·90 (0·24)	0·08 (0·01)
2 μg	123·4 (14·3)	2,581,250 (266,610)	68·4 (8·5)	4·14 (0·65)	0·45 (0·05)	0·03 (0·01)
0·2 μg	83·6 (9·3)	911,250 (96,983)	73·6 (3·9)	5·52 (1·22)	0·55 (0·04)	0·02 (0·01)
DS 1 mg	131·5 (12·2)	934,375 (36,577)	67·1 (7·0)	0·03 (0·02)	0·90 (0·17)	0·09 (0·02)
0·1 mg	83·7 (1·1)	659,375 (31,447)	80·8 (3·2)	0·21 (0·04)	0·84 (0·11)	0·06 (0·02)
PWM 0·3 ml	113·8 (13·9)	1,718,750 (189,949)	80·7 (11·3)	0·56 (0·14)	1·47 (0·18)	0·05 (0·01)
0·03 ml	98·9 (2·9)	984,375 (125,247)	65·8 (3·6)	0·23 (0·11)	1·11 (0·13)	0·05 (0·02)
0·003 ml	74·0 (5·9)	549,375 (48,093)	65·4 (5·5)	0·09 (0·03)	0·88 (0·21)	0·05 (0·01)

Day 4 after injection of AIr, four CBA males per group. DS is dextran sulphate (Serva) and PWM is pokeweed mitogen (Gibco) which was diluted to a standard concentration according to the manufacturer's instructions. 10 mg DS was a lethal dose.

Table 5. RF and antigen-specific IgM and IgG plaque responses at different times after the injection of specific and non-specific AIr

AIr	Time (days)	Total PFC/10^6 spleen cells*		Response as a % of total IgM or IgG			
				IgM			IgG
		IgM	IgG	MIG	Rat IG	SRBC	SRBC
Nil	0	755	0	—	—	—	—
20 μg LPS i.p.	4	129,263	307	63·0	45·1	0·02	3·5
	6	29,593	2,391	>100	40·0	0·1	0·3
	9	15,822	2,172	68·2	45·0	0·03	0
	12	10,894	1,851	87·2	52·2	0·01	0
0·2 ml Klebsiella† i.p.	4	137,027	—	73·8	52·0	0·1	—
	6	60,340	—	78·3	26·0	0·08	—
	9	32,549	—	52·5	5·7	0·03	—
	12	11,594	—	8·8	14·8	0·1	—
10^8 Sheep RBC i.p.	4	13,254	1,356	64·6	24·4	17·4	5·6
	6	5,621	1,183	>100·0	52·0	11·8	30·8
	9	7,519	1,045	56·8	10·2	0·7	26·7
	12	3,233	343	66·2	34·0	0·6	23·0

* Reverse plaques with SRBC coated with IgG preparation of goat anti-(mouse) Fab, with μ- or γ-specific developing sera raised in rabbits. Goat anti-Fab not as efficient for reverse IgM as goat anti-(mouse)-μ, hence the proportion of MIG (RF plaques) seems impossibly high (>100%) in some instances.

† *K. pneumoniae* virulent strain KGP, heat killed, approximately $2·7 \times 10^9$ organisms per ml. Supplied by Dr J. J. Bullen.

Table 6. The effectiveness of different substances as AIr: heterophile RF PFC, in CBA mice

Substance	Dose	PFC/spleen
0·15M NaCl	0·3 ml	0
LPS 0901 B	20 μg	43,500
0901 W	20 μg	68,758
055 B5 B	20 μg	87,561
0127 B8 W	20 μg	99,910
Serratia marcescens	20 μg	21,329
*Pasteurella pestis**	20 μg	28,345
Poly A: poly U	200 μg	0
Poly I: poly C	200 μg	0
PHA (Difco)†	0·2 ml	7,500
PWM (Gibco)†	0·3 ml	94,250
PWM (Gibco)†	0·06 ml	37,250
Neuraminidase (*Vibrio cholerae*)	20 units	2,875
Freund's complete adjuvant (Difco)	0·2 ml	50
Freund's incomplete adjuvant (Difco)	0·2 ml	100
LLA ET18‡	300 μg	150
Con A	100 μg	600
Rabbit anti-(mouse) B cell IgG§	2 mg	250
on 1 mg Sepharose 4B	100 μg	600
NRS IgG on Sepharose 4B	100 μg	100
Sepharose 4B	1 mg	175
Corynebacterium parvum¶	0·2 ml	0

* Gift from Dr D. A. L. Davies.

† Phytohaemagglutinin and pokeweed mitogen concentration recommended by manufacturers.

‡ Gift from Dr E. Lederer.

§ Raised against spleen cells of thymectomized/foetal liver reconstituted mice. Absorbed once with liver and four times with thymus cells.

¶ Burroughs Wellcome, standard preparation, batch P × 289; i.v. injection, all other injections were i.p.

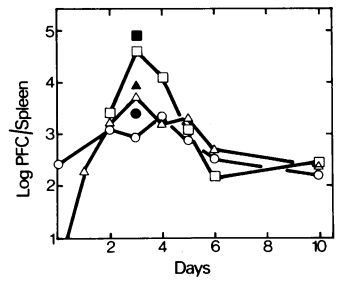

Figure 1. Time–response curves derived from 8 month old CBA males injected with 2×10^9 *B. pertussis* organisms i.p. The solid symbols are the level of PFC after the i.p. injection of 20 μg LPS. ○, SRBC (direct); △, BIG (direct); □, BIG (μ dev.).

tion of two lipoprotein preparations was instrumental in increasing both the level of IgM-producing cells and the proportion of that total which is directed against BIG coated target cells. The significantly higher proportion of anti-BIG RF plaques after the injection of *Salmonella salminitis* lipoprotein may possibly be related to that organism having been grown on a beef broth medium. In this connection it may be relevant to note that in some experiments unrelated to those described here, the injection of a mixture of LPS and small amounts (10–30 μg) of BIG, resulted in a similarly high proportion of anti-BIG plaques.

Dextran sulphate seems to be an AIr which is qualitatively different to LPS (Table 3) in that it stimulates an increase in total IgM and in anti-SRBC producing cells to approximately the same relative degree as LPS,

Table 7. Activation of immune responsiveness by a soluble extract prepared from Schistosomes: stimulation of a heterophile RF response

	PFC/spleen			
Activator	Total IgM	BIG (μ dev.)*	SRBC (direct)	DNP-(direct)†
None	14,220	0	146	—
Schisto‡ 0·1 ml	82,163	38	573	2874
0·5 ml	28,512	700	99	3884
1·0 ml	89,375	3875	850	5900
20 μg LPS (day 2)	111,700	6350	16,305	5998

* Subtraction of residual anti-SRBC PFC in same concentration of anti-μ 'developing' serum.

† Direct anti-SRBC-PFC subtracted; DNP-(rabbit)-Fab, anti-SRBC used to coat indicator RBC with DNP groups (Gift from Dr Marc Feldmann).

‡ Assayed day 4 after i.p. injection of indicated amount of a soluble extract, prepared in a standard manner from *Schistosoma mansoni* adults by Dr Juarez Pinto (Ramalho-Pinto, Goldring, Smithers & Playfair, 1976).

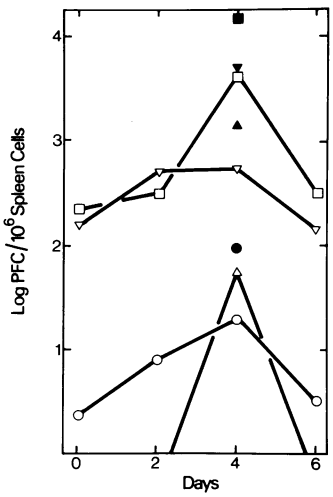

Figure 2. Time–response curves from 8 month old CBA males injected with a soluble antigen preparation from *Schistosoma mansoni*. The solid symbols are the level of PFC reached in mice injected 4 days previously with 20 μg LPS. □■, total IgM; ▽▼, TNP (direct); △▲, BIG (μ dev.); ○●, SRBC (direct).

but does not seem to affect the level of heterophile (anti-BIG) or anti-TNP PFC to any measurable extent. This has been investigated further in the experiment summarized in Table 4, where it can be seen that the failure to activate a heterophile (BIG) RF response cannot be accounted for in terms of dosage thresholds.

When SRBC ('specific' AIr) are injected into CBA mice there is a considerable increase in IgM-producing cells. Since only 17% of the IgM appearing after injection of SRBC are specific for SRBC (Table 5) it follows that most of the 12,000 IgM PFC appearing as the consequence of SRBC injection are producing 'antibody' directed against antigenic sites other than those found naturally on SRBC or, less likely, are producing some kind of aberrant IgM molecule with no antibody activity at all. In this respect therefore it appears that the activation by SRBC has a significant element of non-specificity and therefore similarity to LPS, in the consequences to its injection. The results summarized in Table 5 also demonstrate an increase in total (reverse) IgG producing cells after LPS and SRBC injection. As with total (reverse) IgM-PFC the reverse IgG PFC after SRBC injection exceed the numbers producing antibody against SRBC. Although it has not been possible to demonstrate this definatively, the difference may represent in part IgG PFC (G anti-G). The reverse plaque target reagent for this enumeration was an IgG preparation of a goat-anti-(mouse)-Fab serum used in conjunction with rabbit antisera whose antibody activity is directed against mouse-μ and -γ respectively. This (anti-Fab) reagent is only about 40% as efficient for total IgM detection, as the goat anti-(mouse)-μ reagent used in other experiments described in this paper. Despite its inefficiency it has been possible to show that the level of specificity in the IgG response after SRBC injection is much higher than in the IgM response.

Table 6 lists twenty substances which were screened for their ability to activate heterophile (BIG) RF responses in CBA mice. Apart from lipopolysaccharides from Gram-negative bacteria the only strong activator is pokeweed mitogen with somewhat lesser effects

Table 8. Activation of heterophile RF and other non-specific immune responses in mice infected with *Trypanosoma brucei*

Activator	Time* (days)	PFC/spleen (±SE)† Total IgM	BIG (μ-dev.)	SRBC (direct)	TNP-(direct)
None	—	30,417 (22,928)	17 (17)	133 (77)	1,238 (606)
LPS (20 μg i.p.)	4	—	93,800 (35,699)	9067 (371)	292,670 (17,070)
T. brucei (infection)	8	574,167 (111,844)	6,583 (1,965)	—	27,000 (5,387)
T. brucei (injection)	10	81,667 (15,023)	908 (584)	2775 (743)	5,750 (808)

* Time after injection or infection.
† In (CBA × C57B1/6) F_1 males.

Table 9. Killed trypanosomes act as a non-specific activator of direct IgM responses but fail to stimulate a heterophile RF-response

Activator	PFC/spleen (day 4 response)			
	Total IgM	BIG (μ-dev.)	SRBC (direct)	TNP_{Hi}-(direct)
None	37,750	0	25	21,475
LPS (50 μg)	19,544,250	516,750	33,000	702,000
LPS (50 μg) + 100 μg BIG	10,875,500	540,000	23,250	382,500
*T. brucei**	359,400	0	7,800	124,200

* Approx. 10^8 *Trypanosoma brucei* organisms, irradiated 30 Kr (^{60}Co) immediately prior to i.p. injection.

from PHA and neuraminidase. Figure 1 shows that *Bordetella pertussis* (Bp) organisms are also a good non-specific AIr and that the time course of the response is much the same as that already reported for LPS (Dresser & Popham, 1980).

Table 7 and Figure 2 extend the observations mentioned above for Bp to soluble extracts of *Schistosoma mansoni*, while Tables 8 and 9 show that *Trypanosoma brucei*, either as killed (irradiated) organisms or as an active infection, stimulate an increase in total IgM PFC. Killed *T. brucei*, in common with dextran sulphate, fail to stimulate a heterophile RF response although an active infection appears to be able to do so. The observed difference may be less a reflection of the state of the organism than of the mode of exposure of the mice to its antigens; continuous generation of small quantities of trypanosome antigens versus a single massive shot of material rapidly cleared from the circulation by normal physiological processes.

DISCUSSION

When it was first observed that LPS injected into CBA mice stimulated the appearance of indirect (μ) PFC against bovine IgG (BIG) it seemed possible that this phenomenon was due to contamination of the LPS preparation by traces of BIG antigenic determinants. Two experiments (viz. Tables 2 and 9) certainly suggested that BIG antigens mixed with LPS increased the proportion of IgM-producing cells which are making an immunoglobulin with anti-BIG activity. However, further examination of the data makes it clear that LPS activates the immunocytes in a random (polyclonal) manner, possibly increasing the average avidity of the secreted IgM antibody by selecting for those cells which produce a high affinity antibody for BIG (Dresser & Popham, 1976). This conclusion is based on the ability of all LPS preparations to stimulate anti-BIG RF-PFC, irrespective of source or method of preparation, and also the stimulation of the heterophile RF response by several unrelated AIr, including PWM, PHA, neuraminidase, Bp organisms, Schistosome antigens and infection by *T. brucei*,

It is evident from the data in Tables 3 and 4 that dextran sulphate is a powerful *in vivo* 'mitogen' in that it increases the cellularity of the spleen to the same extent as 20 μg LPS. Nevertheless, examination of the data in these Tables reveals that dextran sulphate does not increase heterophile RF responsiveness while LPS does so in a spectacular manner. Table 4 shows that LPS reduces the proportion of total IgM with (direct) anti-TNP activity, whereas PWM, at least at the highest dose, has the opposite effect. This phenomenon, which has been investigated further, may imply that 'anti-TNP' precursors proliferate more slowly than other IgM precursors.

It has been shown (Dresser & Popham, 1980) that the time–response curves after LPS peak at day (3 or) 4 for all the IgM responses measured. Figures 1 and 2 show very similar cadences for responses stimulated by Bp organisms and for schistosome antigens. It therefore seems highly likely that in this respect the mechanism of action of all non-specific AIr is similar. The rise in total IgM PFC and of heterophile RF PFC in particular, after the injection of parasite antigens (Schistosome) or during an active infection (*T. brucei*) is of some general interest. Related observations have been made by Freeman & Parish (1978) and Rosenberg (1978) during rodent malarial infection and by Kobayakawa *et al.* (1979) during *T. brucei* infection. Keeler, Phillips & Dresser (1979) have noted that

serum from mice kept in conventional conditions contains considerably more (IgM) rheumatoid factor than that of mice kept in clean (specific-pathogen free) conditions. It therefore seems likely that background RF and antibodies against miscellaneous third party antigens (e.g. SRBC, TNP-) may be the result of non-specific (polyclonal) random activation by a wide range of substances derived directly or indirectly from interaction of an individual with its 'normal' environment. The possible significance of this non-specific immune response in relation to autoimmune disease and to specific immune responsiveness is not fully understood at present and awaits positive experimental analysis.

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