IDENTIFICATION OF SOLUBLE Fc RECEPTORS IN MOUSE SERUM AND THE CONDITIONED MEDIUM OF STIMULATED B CELLS

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The ability of cells to bind immunoglobulin (Ig) and immune complexes is mediated by receptors that recognize the Fc portion of Ig. Fc receptors (FcR)[1] have been described on subsets of most cell types of the immune system (reviewed in 1 and 2). The binding of immune complexes to FcR on macrophages and polymorphonuclear leukocytes triggers phagocytosis, cidal mechanisms, and the release of mediators of inflammation (3, 4). Several lines of evidence suggest that FcRs play a role in regulation of the immune response. Fc receptors that are specific for each of the Ig isotypes have been reported (5–8) and, in several cases, isotype-specific FcR are induced on T cells after administration of Ig (9–11). In addition, soluble Ig-binding factors (IBF) that have suppressive or enhancing activity on antibody responses have been described (12–14). These factors may be related to Fc receptors described on cell membranes (15).

The isolation of monoclonal antibodies directed against the Fc receptor specific for IgG2b and IgG1 aggregates ($Fc_{\gamma 2b/\gamma 1}R$) (16) has led to the purification of the receptor (17) and the production of specific polyclonal anti-receptor sera. The monoclonal and polyvalent anti-$Fc_{\gamma 2b/\gamma 1}R$ antibodies have been used to develop a monoclonal sandwich radioimmunoassay with a sensitivity of ~1 fmol of $Fc_{\gamma 2b/\gamma 1}R$ (18). In the studies reported here we have used this radioimmunoassay to study the expression of $Fc_{\gamma 2b/\gamma 1}R$ by murine spleen cells after polyclonal activation in vitro, the release of "soluble" $Fc_{\gamma 2b/\gamma 1}R$ by lipopolysaccharide (LPS)-activated splenocytes, and the presence of $Fc_{\gamma 2b/\gamma 1}R$-like material in mouse serum.

Materials and methods

Animals. CD_1F_1, germ-free CD_1, and their sex- and age-matched controls were obtained from Charles River Breeding Laboratories (Wilmington, MA). A/J, C57BL/6J,

This work was supported by grants AI-141603, AI-2131501, and CA-30198 from the U. S. Public Health Service. J. Unkeless is an American Cancer Society Research Scholar. E. Pure is supported by Damon Runyon–Walter Winchell Fellowship DRG-565.

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; Con A, concanavalin A; FBS, fetal bovine serum; FcR, Fc receptor; $Fc_{\gamma 2b/\gamma 1}R$, receptor for the Fc portion of IgG2b and IgG1; HBSS, Hanks' balanced salt solution; HSB, high-salt buffer; IBF, immunoglobulin-binding factor; LPS, lipopolysaccharide; NP-40, Nonidet P-40; PD, Dulbecco's modified phosphate-buffered saline; RAMIg, rabbit anti-mouse Ig; RIA, radioimmunoassay; SDS, sodium dodecyl sulfate; SDS-PAGE, SDS–polyacrylamide gel electrophoresis.

B6A/J, BALB/c nu/nu and BALB/c nu/+ controls were from The Jackson Laboratories (Bar Harbor, ME). Swiss mice were from the Rockefeller University colony.

Antibodies. Affinity-purified rabbit antibodies against mouse Ig (RAMIg) and μ (R$\alpha\mu$), and rat Ig were generously provided by Dr. E. S. Vitetta (University of Texas Health Science Center, Dallas, TX). Affinity-purified rabbit anti-mouse γ was purchased from Jackson ImmunoResearch Laboratories, Inc. (Avondale, PA). Two monoclonal anti-Thy-1.2 antibodies were used. Anti-Thy-1.2 (IgM_k) is the product of the HO-13.4 hybridoma (19), and anti-Thy-1.2 (rat IgG) is the product of the HO-12.4 hybridoma. The rat anti-Lyt-1 (53.7.313) and anti-Lyt-2 (52.6.72) hybridomas (20) were obtained from Dr. E. S. Vitetta, and purified as previously described (21). Monoclonal anti-$Fc_{\gamma 2b/\gamma 1}R$ antibody, 2.4G2, was purified from hybridoma ascites fluid by ion exchange chromatography on DE52 (Pharmacia Fine Chemicals, Piscataway, NJ). The papain Fab fragment was prepared as described (16) and purified on an LKB TSK 3000 column (LKB Produkter, Bromma, Sweden). A rabbit anti-$Fc_{\gamma 2b/\gamma 1}R$ serum was prepared and adsorbed as described (18).

Depletion of T Cells. T cells were removed by treatment of spleen cells with monoclonal anti-Thy-1.2 (HO-13.4) and baby rabbit serum as a source of complement (C) (Pel-Freez Biologicals, Rogers, AR). Alternatively, cells were treated with a cocktail of rat hybridoma antibodies directed against Thy-1.2 (HO-12.4), Lyt-1, and Lyt-2, followed by rabbit anti-mouse γ chain and C. These treatments abolished the proliferative response to concanavalin A (Con A).

Adherent Cell Depletion. Adherent cells were depleted by passage of spleen cells twice through Sephadex G-10. Alternatively, splenocytes were allowed to adhere to plastic tissue culture dishes for 2 h at 37°C. The nonadherent fraction was replated for an additional 2 h after which the nonadherent cells were harvested by gentle pipetting.

Depletion of B Cells. B cells were removed by panning on RAMIg-coated dishes (22) followed by passage through a nylon wool column (23). LPS-stimulated [^{3}H]thymidine incorporation and Ig secretion of the depleted population was <5% that of the untreated control.

Culture Conditions. Spleen cells were cultured in 96-well microculture plates (Costar, Cambridge, MA) or 75-cm^2 flasks (Corning Glass Works, Corning, NY) at 0.5–1 × 10^6 cells/ml in Hepes-buffered RPMI 1640 supplemented with penicillin (100 U/ml), streptomycin and gentamycin (10 μg/ml), 50 μM 2-mercaptoethanol, and 10% heat-inactivated fetal bovine serum (FBS) (KC Biological Inc., Lenexa, KS). Cultures were incubated in an atmosphere of 7% CO_2 at 37°C. Cells were stimulated with 5 μg/ml Con A (Miles Laboratories, Inc., Elkhart, IN) or 20–50 μg/ml LPS (*Salmonella typhosa*; Difco Laboratories, Inc., Detroit, MI) for the indicated intervals. Proliferation was measured by incorporation of [^{3}H]thymidine added during the last 16 h of culture. J774 cells and S49.1 cells were grown in spinner cultures in Dulbecco's modified Eagle's medium plus 5% FBS and α–minimum essential medium with 10% FBS, respectively. The BCL_1 clone, CW 13.20-3B3 (CW 13.20), was generously provided by Drs. K. Brooks and E. S. Vitetta and maintained as described (24).

Radioiodination of Protein. Protein (<50 μg) was labeled with ^{125}I as previously described (18). However, iodinated $Fc_{\gamma 2b/\gamma 1}R$ was separated from free iodide by dialysis against Dulbecco's modified phosphate-buffered saline (PD).

^{125}I-2.4G2-binding Assay. Cells were washed by centrifugation in Hanks' balanced salt solution (HBSS) containing 1 mg/ml bovine serum albumin (HBSS/BSA). Cells (5–10 × 10^5) were then incubated for 1 h at 4°C with 0.5 μg/ml ^{125}I-2.4G2 Fab (sp act, 5,000 cpm/ng) in 100 μl of HBSS/BSA. Cells were washed extensively in HBSS/BSA and the cell pellets were assayed for radioactivity.

Radioimmunoassays (RIA). Supernatants from cultures of stimulated cells were assayed for the presence of secreted IgM and IgG by a solid phase RIA as previously described (25). $Fc_{\gamma 2b/\gamma 1}R$ present in whole cell Nonidet P-40 (NP-40) lysates, culture supernatants, or serum was detected by a monoclonal sandwich RIA (18). Briefly, aliquots of the cell-free supernatants, cell lysates, or serum were incubated with ^{125}I-2.4G2 Fab (0.5 μg/ml, 5,000 cpm/ng) in a total volume of 50 μl for 1 h at room temperature. The resultant 2.4G2 Fab–antigen complexes were immunoprecipitated with either anti-$Fc_{\gamma 2b/\gamma 1}R$ or

normal rabbit serum bound to heat-killed formaldehyde-fixed *Staphylococcus aureus* (Zymed Laboratories, Burlingame, CA). The bacteria were washed by centrifugation in PD containing 1% NP-40 and 0.2% sodium dodecyl sulfate (SDS) buffer, and the bacterial pellets were assayed for radioactivity. The relative amount of soluble $Fc_{\gamma 2b/\gamma 1}R$ is expressed either by the amount of ^{125}I-2.4G2 Fab precipitated or in comparison with standards of J774 or S49.1 cell lysates, assuming 10^6 and 2×10^5 receptors per cell, respectively.

Preparation of Serum and Plasma. Mice were anesthetized with ether and bled by cardiac puncture. When plasma was prepared, blood was collected in the presence of heparin.

Affinity Purification. Circulating $Fc_{\gamma 2b/\gamma 1}R$ was affinity purified on 2.4G2 Fab–Sepharose 4B immunoadsorbent columns by passing serum three times through the column. The immunoadsorbent was then washed with 10 column volumes of PD, 10 column volumes of a high-salt buffer (HSB) (0.6 M NaCl, 0.0125 M KPO_4, pH 7.4, and 0.02% NaN_3), followed by 10 column volumes of a mixed detergent solution (0.05% NP-40, 0.1% SDS, 0.3 M NaCl, and 10 mm Tris-HCl, pH 8.6), and again with 10 column volumes of HSB and PD. Bound material was then eluted with 1 M acetic acid, dialyzed into ammonium bicarbonate, and lyophilized. The purified protein was iodinated by the iodogen method (26) and analyzed by immunoprecipitation and SDS–polyacrylamide gel electrophoresis (SDS-PAGE).

Immunoprecipitation. Labeled protein in PD was precleared by incubation with *S. aureus* precoated with normal rabbit serum. The unadsorbed protein was then mixed with normal rabbit- or rabbit anti-$Fc_{\gamma 2b/\gamma 1}R$ antibody–precoated, formaldehyde-fixed *S. aureus* and incubated for 1 h at 4°C. The immunoadsorbent was collected by centrifugation, washed twice in HSB, twice with mixed detergent solution, and finally with PD. The pellets were suspended in Neville's SDS-PAGE running buffer (27) and boiled for 5 min and centrifuged at 10,000 *g*.

SDS-PAGE. SDS-PAGE was performed according to the method of Neville and Glassman (27) using 0.75-mm-thick slab gels with a 4–11% polyacrylamide gradient in the running gel. ^{125}I-containing lanes were dried and exposed at −70° on Kodak XR-5 X-omat film using image intensification screens. Apparent molecular weights (M_r) were estimated by comparison with standard proteins (Bio-Rad Laboratories, Richmond, CA).

Results

$Fc_{\gamma 2b/\gamma 1}R$ Expression by LPS-activated Spleen Cells. We have investigated the expression of $Fc_{\gamma 2b/\gamma 1}R$ by murine splenocytes after polyclonal activation by LPS or Con A in vitro for 4–6 d. Compared with untreated spleen cells or spleen cells incubated with Con A, treatment of spleen cells with LPS resulted in an increase in the amount of cell-associated $Fc_{\gamma 2b/\gamma 1}R$ measured by RIA in detergent lysates (Fig. 1*A*). The increase in total cell-associated $Fc_{\gamma 2b/\gamma 1}R$ is reflected by the increased number of $Fc_{\gamma 2b/\gamma 1}R$ on the cell surface of LPS-activated cells, as detected by the binding of ^{125}I-2.4G2 (Fig. 1 and Table I). In contrast, Con A had no effect of $Fc_{\gamma 2b/\gamma 1}R$ expression (Fig. 1).

The increase in cell-associated $Fc_{\gamma 2b/\gamma 1}R$ was accompanied by the appearance in the culture supernatants of soluble material that was detected by the RIA (Fig. 1*B*). The soluble factor must express the determinant recognized by the 2.4G2 Fab as well as at least one additional determinant recognized by the rabbit anti-$Fc_{\gamma 2b/\gamma 1}R$ antiserum, since the assay is dependent upon the immunoprecipitation by polyvalent rabbit anti-$Fc_{\gamma 2b/\gamma 1}R$ serum of ^{125}I-2.4G2 Fab–antigen complexes, and the rabbit antiserum is adsorbed to remove any antibody reactive with 2.4G2 Fab. The soluble factor is thus antigenically related to $Fc_{\gamma 2b/\gamma 1}R$ and will be referred to as soluble $Fc_{\gamma 2b/\gamma 1}R$.

The soluble $Fc_{\gamma 2b/\gamma 1}R$ was not removed from the culture supernatant by

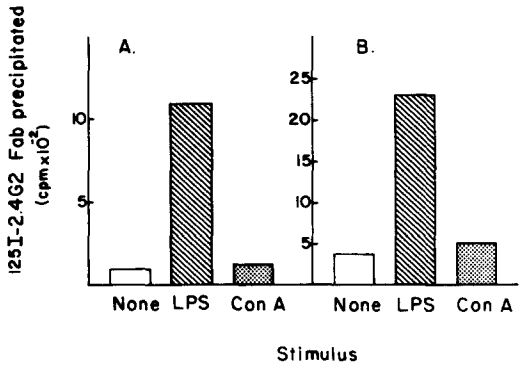

FIGURE 1. LPS-induced spleen cell–associated and soluble $Fc_{\gamma 2b/\gamma 1}R$. CD_2F_1 spleen cells were cultured at 5×10^5/ml in 75-cm^2 flasks for 5 d with no stimuli (open bars), 20 μg/ml LPS (hatched bars), or 5 μg/ml Con A (stippled bars). Cells and cell-free supernatants were harvested at day 5 and cells were lysed in NP-40 at 1×10^7 cell equivalents per milliliter. (*A*) Results of assaying 1×10^5 cell equivalents of cell lysates. (*B*) Radioimmunoassay results obtained with 10 μl of culture supernatant in a representative experiment.

TABLE I
Kinetics of LPS-induced $Fc_{\gamma 2b/\gamma 1}R$

Cells	$Fc_{\gamma 2b/\gamma 1}R$ per cell ($\times 10^{-4}$)			
	Day	LPS	Cell surface	Soluble
T-depleted spleen*	0	+	1.47	NA‡
	1	+	0.95	ND§
	2	+	3.14	2.2
	3	+	2.03	2.3
	4	+	6.07	2.4
	6	+	17.3	47.0
BCL_1 CW13.20	2	−	20.0	ND
		+	120.0	ND
	4	−	20.0	20.0
		+	32.4	145.0

* CD_2F_1 spleen cells were depleted of T cells by anti-Thy-1.2 and C as described in Materials and Methods.
‡ Not applicable.
§ Below the level of detectability.

centrifugation at 100,000 *g* for 90 min or by 100,000 *g* centrifugation followed by filtration through a 0.45 μm filter, which resulted in 88 and 83% recovery, respectively (data not shown). These results suggest that the soluble $Fc_{\gamma 2b/\gamma 1}R$ is not associated with membrane fragments.

LPS-induced $Fc_{\gamma 2b/\gamma 1}R$ Is Associated with B Cells. The cell population responding to LPS with elevated $Fc_{\gamma 2b/\gamma 1}R$ expression was examined by depleting adherent cells and/or by killing T cells with antibody and complement (C). T-depleted spleen cells responded to LPS as efficiently as whole spleen cell cultures with respect to synthesis of soluble and cell-associated $Fc_{\gamma 2b/\gamma 1}R$ (Table II). Similarly, spleen cells depleted of adherent cells by passage through G-10 Sephadex responded to LPS by producing increased cell-associated and soluble $Fc_{\gamma 2b/\gamma 1}R$

TABLE II
Effect of T Cell Depletion on LPS-induced FcR

Exp.	Culture supernatants (FcR/cell recovered, $\times 10^{-4}$)				Cell-associated FcR (FcR/cell recovered, $\times 10^{-4}$)			
	Untreated		T depleted		Untreated		T depleted	
	None	LPS	None	LPS	None	LPS	None	LPS
1	ND	0.82	0.16	0.96	NT	NT	NT	NT
2	1.3	12.0	1.1	11.0	NT	NT	NT	NT
3	0.7	7.2	0.5	4.8	NT	NT	NT	NT
4	ND	1.3	ND	2.6	0.4	3.1	0.6	3.0
5	ND	1.0	ND	0.7	0.1	5.5	2.2	20.0

Con A proliferative responses were inhibited >90% by anti-Thy-1.2 + C or by anti-Thy-1 + anti-Lyt-1 + anti-Lyt-2 followed by anti-rat-γ and C treatment. The number of FcR per cell was calculated by comparing the amount of ^{125}I-2.4G2 Fab precipitated compared with a standard curve obtained with J774 or S49.1 cell lysates, assuming 10^6 and 10^5 receptors per J774 and S49.1, respectively. The limit of the radioimmunoassay is 2×10^5 receptors per cell; all samples below our level of detection are represented by ND. NT, not tested.

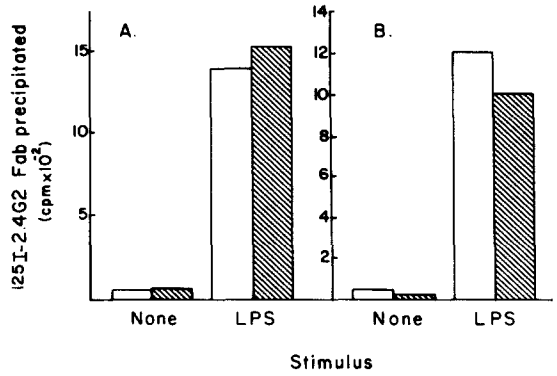

FIGURE 2. Effect of adherent cell depletion of the LPS-induced $Fc_{\gamma 2b/\gamma 1}R$ response. Spleen cells were passed twice through Sephadex G-10 (hatched bars) and then cultured as in Fig. 1 in parallel with untreated spleen cells (open bars). Cell lysates (*A*) (2.5×10^4 cell equivalents) and culture supernatants (*B*) (5 μl) were assayed as in Fig. 1.

(Fig. 2). Finally, we prepared enriched B cell populations by successive depletion of T cells and adherent cells and found that neither T cells nor adherent cells were required for this response (Fig. 3). To confirm this, we tested spleen cells depleted of B cells by panning on anti-Ig–coated dishes followed by passage through a nylon wool column. The B cell–depleted population was unable to proliferate or secrete Ig in response to LPS (data not shown), and stimulation of these cells with LPS had no effect on $Fc_{\gamma 2b/\gamma 1}R$ measured in cell lysates or cell-free supernatants (Fig. 4).

We have obtained similar data using a cloned B cell tumor line, BCL_1 CW13.20-3B3. These cells respond to LPS, as do normal B cells, by differentiating into IgM-secreting cells (24). The BCL_1 cell line also responds to LPS with increased surface $Fc_{\gamma 2b/\gamma 1}R$ and soluble $Fc_{\gamma 2b/\gamma 1}R$ (Table I).

The increase in cell-associated $Fc_{\gamma 2b/\gamma 1}R$ on spleen cells stimulated with LPS preceeds the detection of soluble $Fc_{\gamma 2b/\gamma 1}R$ in cell-free culture supernatants

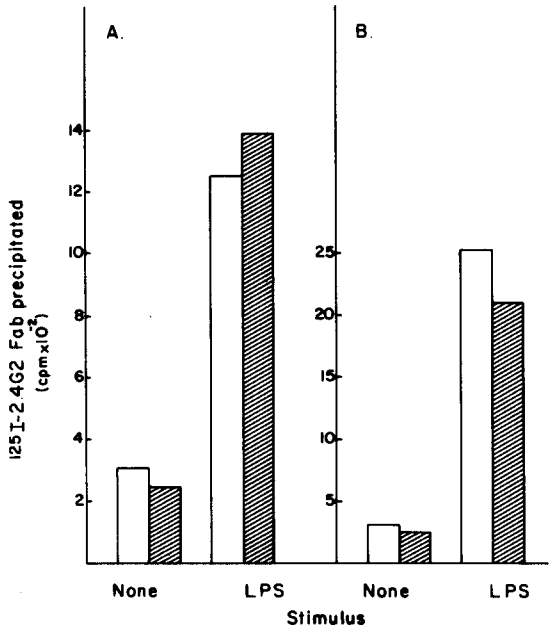

FIGURE 3. Induction of $Fc_{\gamma 2b/\gamma 1}R$ by LPS treatment of B cell–enriched spleen cells. Spleen cells were depleted of T cells and adherent cells as described above and the B cell population (hatched bars) cultured as in Fig. 1 in parallel with untreated spleen cells (open bars). Cell lysates (5×10^4 cell equivalents) and culture supernatants (10 μl) were assayed as in Fig. 1.

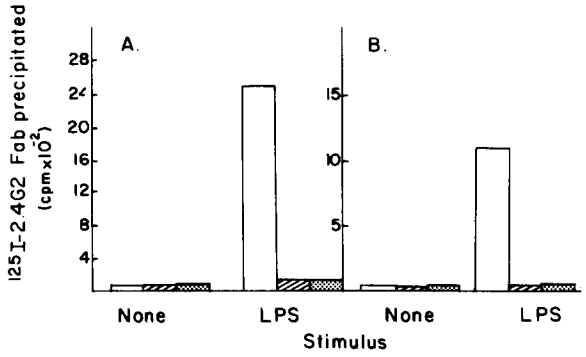

FIGURE 4. Effect of B cell depletion on LPS-induced $Fc_{\gamma 2b/\gamma 1}R$ expression. Splenic B cells were depleted by panning on RAMIg-coated dishes (solid bars) or by panning followed by passage through nylon wool (hatched bars) and were then cultured in parallel with untreated spleen cells (open bars) as described in Fig. 1. 1×10^5 cell equivalents of the lysates or 5 μl of culture supernatant were assayed for $Fc_{\gamma 2b/\gamma 1}R$ by the monoclonal radioimmunoassay.

(Table I). The increase in cell surface $Fc_{\gamma 2b/\gamma 1}R$ was apparent by day 2 and continued to increase through day 5. In contrast, levels of soluble $Fc_{\gamma 2b/\gamma 1}R$ did not significantly increase until day 4 or 5. The cell viability ranged from 65 to 85% throughout these experiments. Similar observations were made for the BCL_1 cell line, except that the kinetics were more rapid (Table I). On the second day after LPS stimulation, a sixfold increase in cell surface $Fc_{\gamma 2b/\gamma 1}R$ was observed but no soluble $Fc_{\gamma 2b/\gamma 1}R$ was detected. Interestingly, at 4 d, the amount of surface

TABLE III
FcR in Pooled Mouse Serum

Strain	Sex	Preparation	Soluble FcR concentration (M^{-9})*
BALB/c	F	Unadsorbed	2.4
		2.4G2 Fab adsorbed‡	ND
		Rat Fab adsorbed‡	2.2
B6AJ	F	Unadsorbed	2.0
C3H/HeJ	F	Unadsorbed	1.7
		Plasma	1.5
$CB6F_1$	F	Unadsorbed	11.8
CD_2F_1	M	Unadsorbed	7.0
		2.4G2 Fab adsorbed	ND
		Rat Fab adsorbed	6.6
A/J	M	Unadsorbed	1.0
Swiss	F	Unadsorbed	9.0
		2.4G2 Fab adsorbed	ND
		Rat Fab adsorbed	8.6

Serum was prepared from at least five mice 2–4 mo old.
* FcR concentration was calculated by determining the number of J774 cell equivalents per sample and assuming 10^6 receptors per J774 cell and an M_r of 50,000 based on the M_r of purfied FcR from cell lysates. Not detectable (ND) indicates the sample contained undectable levels of FcR in 20 μl (10 times the volume assayed for positive samples).
‡ Serum was adsorbed by incubation with 2.4G2 Fab or normal rat Fab coupled to Sepharose 4B, each at 2 mg protein/ml Sepharose for 1 h at 4°C.

FcR declined compared with day 2, and the amount of soluble $Fc_{\gamma 2b/\gamma 1}R$ increased sevenfold in comparison with controls. The viability of the cell line was >90% in both control and LPS-stimulated cultures.

In Vivo Analog of Soluble $Fc_{\gamma 2b/\gamma 1}R$. The observation of $Fc_{\gamma 2b/\gamma 1}R$-cross-reactive material in the cell-free culture supernatants of LPS-activated splenocytes prompted us to search for an in vivo equivalent of this molecule. Serum or plasma was prepared from blood obtained from mice by cardiac puncture and was assayed for soluble $Fc_{\gamma 2b/\gamma 1}R$ by RIA. As shown in Table III, pooled serum and plasma from all strains tested contained soluble $Fc_{\gamma 2b/\gamma 1}R$ at a level of 10^{-9} to 10^{-8} M. This material was quantitatively removed by passage over a 2.4G2 Fab–Sepharose column but not over a control rat Fab column (Table III).

To assess if the level of $Fc_{\gamma 2b/\gamma 1}R$ in serum could be correlated with the state of activation of the immune system, we assayed serum from neonatal and germ-free mice. The quantity of soluble $Fc_{\gamma 2b/\gamma 1}R$ in serum increased with the age of the animal (Table IV). The only adult mice that had low levels of soluble $Fc_{\gamma 2b/\gamma 1}R$ in their serum were germ-free animals (Table IV). To further analyze the significance of circulating $Fc_{\gamma 2b/\gamma 1}R$, we quantitated soluble serum $Fc_{\gamma 2b/\gamma 1}R$ in mice with immunologic defects, including T cell–deficient nude mice, autoim-

TABLE IV

Serum FcR in Individual Germ-free Mice and Mice with Immune Defects

Strain	Defect	*n*	Sex	Age	Serum FcR (M [$\times 10^{-9}$] ± SEM)
				mo	
CD_1	Control	6	F	2.5	2.5 ± 0.5
	Germ-free	5	F	2.5	0.5 ± 0.08
BALB/c	nu/+	10	F	2.5	1.7 ± 0.83
	nu/nu	10	F	2.5	3.0 ± 0.23
MLR 1pr/1pr	Autoimmune	5	F	1	3.9 ± 1.2
		5	F	5	10.6 ± 1.5
MRL/N	Autoimmune	5	F	1	0.9 ± 1.1
		5	F	5	5.2 ± 1.5
		5	F	14	6.2 ± 1.6
BXSB	Autoimmune	5	M	1	3.8 ± 0.8
		5	M	5	3.2 ± 0.5
		5	F	1	1.0 ± 0.8
		5	F	4	2.5 ± 0.8
NZB	Autoimmune	5	F	1	0.8 ± 0.5
		5	F	10	3.3 ± 0.6
NZB × NZW		5	F	1	3.0 ± 0.65
		5	F	7	1.8 ± 0.65
C57 1pr/1pr	Immunoproliferative	5	F	1	4.7 ± 2.15
		5	F	10	6.6 ± 0.9
		5	M	1	2.8 ± 1.3
		5	M	10–14	5.8 ± 1.6
C3H 1pr	Immunoproliferative	5	F	1	0.76 ± 0.3
		5	F	10	2.4 ± 0.8
C3H	Control	5	F	1	1.6 ± 0.3
		5	F	7	4.2 ± 0.8
C57	Control	5	F	2	6.4 ± 1.5
		5	F	7	6.3 ± 0.96
BALB/c	Control	5	F,M	1	2.6 ± 1
		5	F	7	6.4 ± 0.8

* Serum was prepared from individual mice.

‡ Concentration of soluble FcR was determined as in Table III and expressed as the average ± standard error of the mean for each group.

mune mice (NZB and NZB × NZW), and mice with autoimmune immunoproliferative disorder (MRL 1pr/1pr) (Table IV). BALB/c nude mice (BALB/c nu/nu) were found to have levels of soluble $Fc_{\gamma 2b/\gamma 1}R$ comparable to that found in their H-2, sex- and age-matched controls (BALB/c nu/+). Adult mice with autoimmune diseases were found to have levels of soluble $Fc_{\gamma 2b/\gamma 1}R$ comparable to that found in their H-2 sex- and age-matched controls. However, MRL, C57BL/6-congenic for the lpr gene of MRL, and BXSB mice, which display

TABLE V
Ig-binding Activity of Serum $Fc_{\gamma 2b/\gamma 1}R$

	$Fc_{\gamma 2b/\gamma 1}R$ (J774 cell equivalents/ml [$\times 10^{-5}$])	
	Exp. 1	Exp. 2
Unadsorbed	19.8	48.0
Rabbit IgG–Sepharose	3.2	4.8
Rabbit $(Fab')_2$–Sepharose	12.1	22.0
BSA-Sepharose	NT	19.0
Percent nonspecific adsorption*	39	54
Percent specific adsorption‡	74	78

* Nonspecific adsorption = [100 − (rabbit $(Fab')_2$–Sepharose)/(Unadsorbed)]/× 100.
‡ Specific adsorption = [100 − (rabbit IgG–Sepharose)/(rabbit $(Fab')_2$–Sepharose)] × 100.

marked hypergammaglobulinemias, have adult levels of serum $Fc_{\gamma 2b/\gamma 1}R$ at 1 mo, whereas normal mice do not reach adult levels until ~2 mo. Male BXSB mice show this high level of $Fc_{\gamma 2b/\gamma 1}R$ at 1 mo but females do not. The autoimmune disease of this strain is more prominent and appears earlier in males compared with females (28).

Ig-binding Activity of Serum FcR-like Material. To determine whether the molecule(s) detected in serum that are antigenically related to $Fc_{\gamma 2b/\gamma 1}R$ are also functionally related to the receptor we investigated the Ig-binding capacity of this material. Mouse serum was adsorbed on normal rabbit IgG or the $F(ab')_2$ fragment of rabbit IgG or BSA coupled to Sepharose 4B. The adsorbed serum was then tested in the RIA for $Fc_{\gamma 2b/\gamma 1}R$. As shown in Table V, 39–54% of the 2.4G2-reactive material was nonspecifically adsorbed to BSA-Sepharose or the $F(ab')_2$ fragment of IgG. However, comparing the adsorption to the intact IgG-Sepharose with that adsorbed to the $F(ab')_2$ fragment, ~75% of the material was specifically bound to the intact IgG, demonstrating its binding activity for the Fc portion of Ig.

Affinity Purification and Characterization of Serum $Fc_{\gamma 2b/\gamma 1}R$-like Molecules. Mouse serum (or rat serum as a control), was adsorbed to 2.4G2 Fab–Sepharose, washed, and the bound protein eluted with 1 M acetic acid as described in Materials and Methods. The eluate was dialyzed, lyophilized, and then dissolved in PD. The mouse and rat proteins were radioiodinated in parallel and aliquots of each were then precleared with normal rabbit Ig–Sepharose and immunoprecipitated with rabbit anti-mouse $Fc_{\gamma 2b/\gamma 1}R$ (Fig. 5, *B* and *C*) or normal rabbit antiserum (Fig. 5, *A* and *D*) adsorbed to fixed *S. aureus*. The immunoprecipitates were subjected to electrophoresis on a 4–11% gradient SDS-polyacrylamide gel and analyzed by autoradiography. A polypeptide species of M_r 48,000 was immunoprecipitated from the affinity-purified mouse serum protein by the rabbit anti-$Fc_{\gamma 2b/\gamma 1}R$ (Fig. 5, *B*), whereas this 48,000 M_r protein was absent in the normal rabbit serum control (Fig. 5, *A*). Furthermore, no 48,000 M_r protein was immunoprecipitated by the rabbit anti-$Fc_{\gamma 2b/\gamma 1}R$ serum from rat serum affinity-purified on 2.4G2 Fab–Sepharose. Rat macrophages lack the 2.4G2

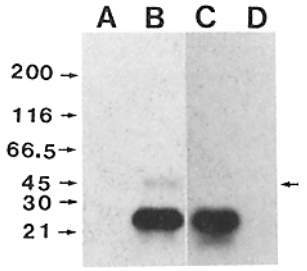

FIGURE 5. Immunoprecipitation of circulating $Fc_{\gamma 2b/\gamma 1}R$. 2.4G2 Fab affinity-purified mouse (*A* and *B*) or rat (*C* and *D*) serum proteins were precleared with normal rabbit sera adsorbed to *S. aureus*. The unadsorbed material was then immunoprecipitated with normal rabbit sera (*A* and *D*) or rabbit anti-$Fc_{\gamma 2b/\gamma 1}R$ sera (*B* and *C*) bound to *S. aureus*. The precipitates were collected by centrifugation and washed as described in Materials and Methods. The precipitates were then subjected to electrophoresis on a 4–11% SDS-polyacrylamide gel and analyzed by autoradiography. The numbers indicated by arrows on the left represent the M_r ($\times 10^{-3}$) of standard proteins run in parallel. The arrow at the right marks the 48,000 M_r species that is specifically immunoprecipitated from the affinity-purified mouse serum proteins.

determinant and rat serum does not react in the RIA for murine $Fc_{\gamma 2b/\gamma 1}R$ (E. Pure, unpublished results). The dominant 23,000 M_r band precipitated by the rabbit anti-mouse $Fc_{\gamma 2b/\gamma 1}R$ from both preparations (Fig. 5, *B* and *C*) is 2.4G2 Fab stripped from the immunoadsorbent column that reacts with anti-rat IgG reagents (data not shown). The 23,000 M_r material is the dominant iodinated species in the partially purified receptor, and the rabbit anti-$Fc_{\gamma 2b/\gamma 1}R$ antiserum, despite adsorption, still immunoprecipitates some of this material.

Discussion

Polyclonal activation of murine splenocytes with LPS in vitro results in an increased expression of $Fc_{\gamma 2b/\gamma 1}R$ and in the appearance of a soluble non-membrane-associated molecule that is antigenically related to the $Fc_{\gamma 2b/\gamma 1}R$. The increase in cell-associated and soluble $Fc_{\gamma 2b/\gamma 1}R$ after stimulation of spleen cells with LPS is attributable primarily to B cells. The kinetics of appearance of the soluble $Fc_{\gamma 2b/\gamma 1}R$, in both spleen cells and BCL_1 cells stimulated by LPS, lags somewhat behind the increase in cell-associated $Fc_{\gamma 2b/\gamma 1}R$. It is possible that Fc receptors may be synthesized in two distinct forms, one that is released as soluble protein and a second anchored in the plasma membrane. Precedents for such soluble and membrane-bound alternatives exist for Ig (29, 30) and H-2 (31, 32) molecules in which different exons encoding the carboxyl termini of the two forms are preferentially selected during RNA processing. Another possible mechanism is that the soluble $Fc_{\gamma 2b/\gamma 1}R$ and the membrane forms are products of closely related genes. Alternatively, the soluble form may be derived from the cell surface molecule by proteolysis.

The in vivo analog of the soluble $Fc_{\gamma 2b/\gamma 1}R$ is probably the 2.4G2-cross-reactive material that we detect in mouse serum by RIA. Based on titrations of $Fc_{\gamma 2b/\gamma 1}R$ in cell lysates by RIA, we estimate that circulating $Fc_{\gamma 2b/\gamma 1}R$ is present at 1×10^{-9} to 1×1^{-8} M in serum or plasma. Soluble $Fc_{\gamma 2b/\gamma 1}R$ was present in the serum of all male and female mice ranging in age from 3 d to 18 mo and was not restricted to any particular H-2 haplotype. That the quantity of $Fc_{\gamma 2b/\gamma 1}R$ in T

cell–deficient nude mice was not markedly different from that found in the normal controls supports our in vitro data that mature T cells are not required for the production of soluble $Fc_{\gamma 2b/\gamma 1}R$. In agreement with Khayat et al. (33), we find the level of circulating $Fc_{\gamma 2b/\gamma 1}R$ generally higher in adult compared with neonatal mice. Interestingly, the only adult mice that differed markedly in their level of circulating $Fc_{\gamma 2b/\gamma 1}R$ were germ-free mice, which had very low or undetectable levels of $Fc_{\gamma 2b/\gamma 1}R$ in their serum compared with the sex- and age-matched controls. This suggests that expression of soluble $Fc_{\gamma 2b/\gamma 1}R$ in normal mouse serum may also be modulated by activation of the immune system.

The circulating $Fc_{\gamma 2b/\gamma 1}R$ from serum that was affinity-purified on a 2.4G2 Fab–immunoadsorbent column and immunoprecipitated with rabbit anti-$Fc_{\gamma 2b/\gamma 1}R$ has an M_r of 48,000 in SDS-PAGE, and migrates in gels as a reasonably narrow species. In contrast, the cell-associated receptor isolated by the same method from BCL_1 (E. Pure, unpublished results) or mouse macrophage cell lines (17) was considerably more diffuse, ranging from 47,000 to 68,000 M_r. A soluble FcR has been isolated by affinity chromatography from metabolically labeled $P388D_1$ cells by Loube and Dorrington (34). The purified protein was a clearly defined doublet in the M_r range 55,000–65,000. The circulating form of the $Fc_{\gamma 2b/\gamma 1}R$ described here is intriguingly similar with respect to molecular weight to the lower molecular weight species isolated from cells. However, further biochemical studies are needed to clarify the relationships among the different molecules.

The role of Fc receptors in the regulation of humoral immune responses and the relationship of soluble Ig-binding factors (IBF) to membrane-bound Fc receptors or the soluble $Fc_{\gamma 2b/\gamma 1}R$–like material described is not yet clear, but the emerging theme is that IBF from T cells regulate B cell Ig synthesis and that IBF may represent a soluble form of T cell Fc receptor. Both we and Khayat (33) have shown that the circulating $Fc_{\gamma 2b/\gamma 1}R$ is capable of binding to IgG. Fc receptors specific for various Ig classes have been described on T and B cells (5, 10, 11, 16, 35–37). Indeed, it has been shown (14, 38) that T cells bearing FcR regulate the synthesis of IgM and IgG antibodies, in part by secretion of soluble T cell factors that bind IgG. Others have shown (13, 39) that T cells bearing Fc receptors specific for IgG (FcR) regulate IgE antibody production via the release of T cell factors having affinity for IgE. Future studies will therefore need to address the relation and function of B cell–derived soluble $Fc_{\gamma 2b/\gamma 1}R$ and T cell–derived IBF.

Summary

We have evaluated the expression of surface $Fc_{\gamma 2b/\gamma 1}R$ by lipopolysaccharide (LPS)-activated murine spleen cells, the release of soluble $Fc_{\gamma 2b/\gamma 1}R$ by activated spleen cells, and the presence of circulating $Fc_{\gamma 2b/\gamma 1}R$ in mouse serum. LPS activation of murine spleen cells and a cloned B cell line, BCL-1 CW 13.20-3B3, resulted in a 5–10-fold increase in surface $Fc_{\gamma 2b/\gamma 1}R$ and the concominant appearance in the culture medium of a soluble molecule that is antigenically related to the $Fc_{\gamma 2b/\gamma 1}R$. The increase in cell-associated and soluble $Fc_{\gamma 2b/\gamma 1}R$ after LPS activation is attributable primarily to B cells.

Circulating $Fc_{\gamma 2b/\gamma 1}R$ was also detected in normal mouse serum at a concentra-

tion of 10^{-9} to 10^{-8} M. Levels of circulating $Fc_{\gamma 2b/\gamma 1}R$ increased with the age of the animals, and were low in adult germ-free mice and elevated in young mice with certain autoimmune diseases. The circulating $Fc_{\gamma 2b/\gamma 1}R$ bound to IgG-Sepharose, and was partially purified by affinity chromatography on 2.4G2 Fab–Sepharose. After radiolabeling and immunoprecipitation with rabbit anti-$Fc_{\gamma 2b/\gamma 1}R$ serum, one component, of M_r 48,000, was detected.

We thank Dr. E. S. Vitetta (University of Texas Health Science Center, Dallas, TX) for the antibodies she generously provided. The serum from autoimmune mice was the generous gift of Dr. A. Theofilopoulos (Scripps Clinic and Research Foundation, La Jolla, CA). We thank Drs. R. Steinman, Z. Cohn, and D. Portnoy for helpful discussions throughout this work and critical review of the manuscript. We thank Mrs. G. Silvestri for preparation of the manuscript.

Received for publication 6 September 1984.

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