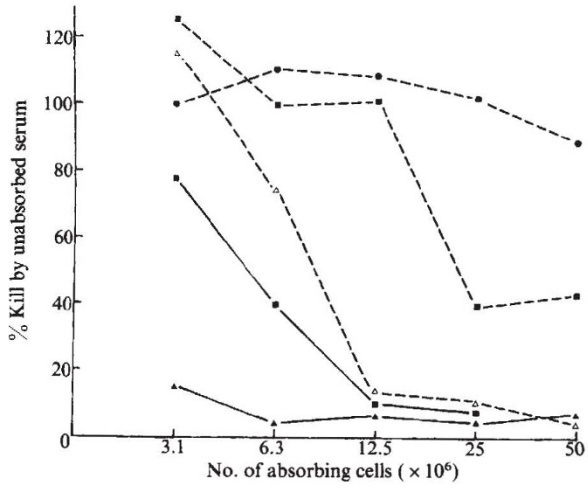

Fig. 1 Absorption analysis of the Ly surface membrane phenotype of hybrids. Preparation, testing and if necessary absorption of Ly sera and rabbit complement was as described previously[23]. Sera were absorbed at the last dilution giving plateau lysis of appropriate thymocyte targets. Aliquots (50 μl) of anti-Ly-1.1 (——) anti-Ly-1.2 (– – –), or anti-Ly-2.1 (data not shown) were absorbed for 1 h at room temperature with $3–50 \times 10^6$ tissue culture grown BW5147 (●), S1.41 (■), S1.34 (data not shown), or freshly prepared CBA (▲) or B10 thymocytes (△). Absorbed sera were assayed by a microcytotoxic test on CBA or B10 thymocytes. 2 μl antiserum was added to 2 μl cell suspension (2,000 cells in Hank's BSS + 5% FCS) in the wells of a microtitre plate (Falcon microtest 3034), and incubated at 37°C for 15 min. One drop of medium was then added and the plate refrigerated for 10 min. The supernatant was then removed by inverting and flicking the plate; 2 μl 1/12 rabbit complement was added and the plate incubated at 37°C for 30 min. A drop of Trypan blue was then added, the plate again refrigerated and flicked, and a further drop of medium added for counting. For each antiserum, positive and negative control absorptions with appropriate thymocytes were carried out in each experiment. For simplicity, the negative control absorptions and negative results obtained with BW5147, S1.41 and S1.34 have been omitted from the figure, except for the example of anti-Ly-1.2 absorbed with BW5147. Other negative results showed essentially the same pattern.

The secreted suppressor factor of the S1-41 hybridoma, SF S1-41 showed no change in function or specificity over a period of 14 months. SF S1-41 has retained all the characteristics of conventional antigen-specific SF, carrying in the same molecule, antigen combining site, Ia (I-J) coded determinants and 'constant' region and 'idiotype-like' determinants of CBA SF_{KLH}. The latter are defined respectively by rabbit and mouse antisera raised against mouse SF[17], and the determinants recognised by the sera have been termed 'constant' and 'idiotypic' or 'variable' by analogy with those parts of conventional Ig. It should be emphasised, however, that there is no evidence for the presence of conventional Ig determinants on either CBA SF_{KLH} (ref 16) or SF S1-41. Furthermore, S1-41 cells bear only T-cell markers Thy-1, Ly-1 and Ly-6, and do not carry surface Ig (unpublished). The characteristics of other specific and non-specific suppressor hybridomas and their products are under study as is the *in vivo* effect of the S1-41 hybridoma.

Our results show that functional T-cell hybrids can be produced by standard techniques and that the established hybrids are stable as judged by surface membrane phenotype and the functional properties of their secreted products. Such hybrids will be extremely useful in the more refined characterisation of the secreted products of T cells of various types, as unlimited cell numbers can be produced and the titre of SF produced by the hybrids is much higher than that of conventional factors.

We thank Ms Deidre Hayton for assistance in testing the SF, and Drs M. B. Rittenberg for KLH, P. H. Maurer for GAT and E. Mozes for (T, G)-A-L. This work was supported by the ICRF, US PHS grant A1-13145-02, and the MRC. S.K., P.V. and A.T. were supported by EMBO fellowships.

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Received 2 May; accepted 14 June 1978.

1. Feldmann, M. *J. exp. Med.* **136,** 737–760 (1972).
2. Munro, A. & Taussig, M. J. *Nature* **256,** 103–106 (1975).
3. Tada, T., Taniguchi, M. & Tokushisa, T. in *Ir Genes and Ia Antigens* 517–527 (Academic, New York, 1978).
4. Takemori, T. & Tada, T: *J. exp. Med.* **142,** 1241–1253 (1975).
5. Kapp, J. A., Pierce, C. W., Dela Croix, F. & Benacerraf, B. J. *J. Immun.* **116,** 305–309 (1976).
6. Kontiainen, S. & Feldmann, M. *Eur. J. Immun.* **7,** 310–314 (1977).
7. Howie, S. & Feldmann, M. *Eur. J. Immun.* **7,** 417–420 (1977).
8. Schimpl, A. & Wecker, E. *Nature new Biol.* **237,** 15–17 (1972).
9. Armerding, D. & Katz, D. H. *J. exp. Med.* **140,** 19–37 (1974).
10. Pierce, C. W. & Kapp, J. A. *Contemp. Top. Immun.* **5,** 91–143 (1976).
11. Köhler, G. & Milstein, C. *Eur. J. Immun.* **6,** 511–519 (1976).
12. Pontecorvo, G. *Somat. cell. Genet.* **1,** 397–400 (1977).
13. Goldsby, R. A., Osborne, B. A., Simpson, E. & Herzenberg, L. A. *Nature* **267,** 707–708 (1977).
14. Köhler, G., Lefkovits, I., Elliot, B. & Coutinho, A. *Eur. J. Immun.* **7,** 758–761 (1977).
15. Hämmerling, G. *Eur. J. Immun.* **7,** 743–746 (1977).
16. Torano, A., Kontiainen, S. & Feldmann, M. (in preparation).
17. Kontiainen, S. & Feldmann, M. (in preparation).
18. Kontiainen, S. & Feldmann, M. *J. exp. Med.* **147,** 110–122 (1978).
19. Kontiainen, S. & Feldmann, M. *Eur. J. Immun.* **6,** 296–301 (1976).
20. Kontiainen, S. & Feldmann, M. *Nature new Biol.* **245,** 285–286 (1973).
21. Kontiainen, S. & Feldmann, M. *Scand. J. Immun.* **4,** 121–128 (1975).
22. March, S. C., Parikh, I. & Cuatre Casas, P. *Analyt. Biochem.* **60,** 149–152 (1974).
23. Feldmann, M., Beverley, P. C. L., Dunkley, M. & Kontiainen, S. *Nature* **258,** 614–616 (1975).
24. Pickel, K. & Hoffman, M. K. *J. exp. Med.* **145,** 1169–1175 (1977).
25. Feldmann, M., Beverley, P. C. L., Woody, J. & McKenzie, I. F. C. *J. exp. Med.* **145,** 793–801 (1977).

Most IgM-producing cells in the mouse secrete auto-antibodies (rheumatoid factor)

IN the mouse the injection of bacterial lipopolysaccharide (LPS) results in the appearance of very large numbers of IgM globulin-secreting cells[1]. Furthermore, it has been shown that LPS induces B lymphocytes, both *in vivo*[2,3] and *in vitro*[4–6] to secrete IgM antibodies to a wide range of seemingly unrelated antigens. It was reported recently that about 8% of all IgM globulin-secreting cells arising as a consequence of LPS injection into CBA mice secrete an antibody-like molecule with specificity for a heterospecific (bovine) IgG (BIG) molecule[1]. It was suggested that such IgM molecules were reminiscent of a rheumatoid factor, although at that time

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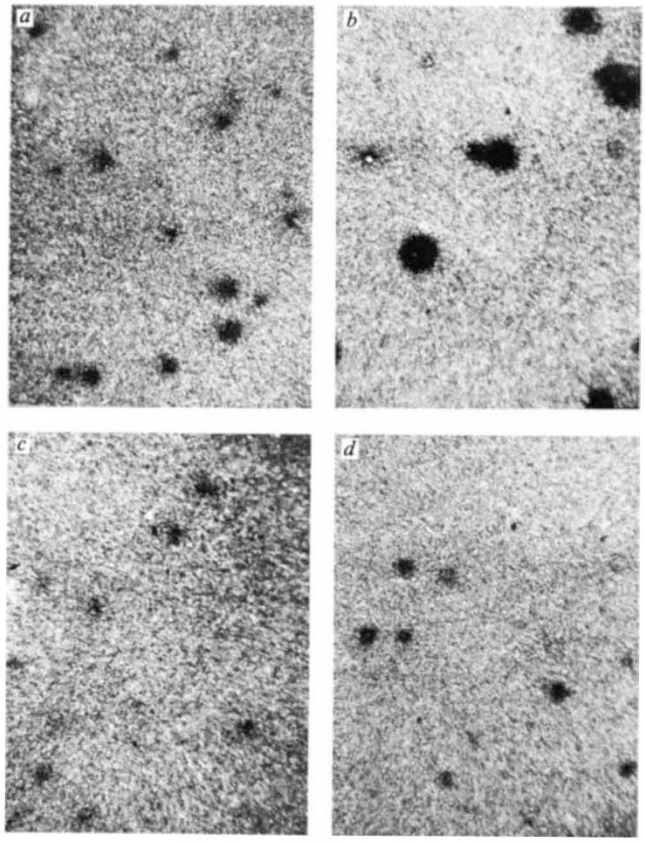
plaque-forming cells (PFC) had not been demonstrated using mouse IgG (MIG) as the target antigen. I have now been able to demonstrate the presence of auto-(anti-MIG)-antibody-forming cells. About half (35–75%) of all IgM-secreting cells appearing after both mitogen and immunogen injection into mice produce IgM globulin which is not specific for the inducing immunogen, but which does have specificity for self-IgG. The description by Steel and Cunningham[7] of a large proportion of IgM-PFC having specificity for normally hidden (syngeneic) erythrocyte antigens, supports a view that a majority of both mitogen and immunogen stimulated IgM-PFC are producing auto-antibodies. Although a mouse rheumatoid-like IgM with binding activity against antigen-bound (syngeneic) IgG was described previously[8], until now attempts to develop a haemolytic plaque assay for autologous mouse M-anti-G have failed. After unsuccessful attempts involving various forms of mouse IgG, partially denatured mouse IgG and also a procedure used successfully in the rabbit[9], a successful method has been developed. An account of some of the results from experiments using this methodology is presented here.

Figure 1 illustrates that mouse auto-immune (M-anti-G) haemolytic plaques are generally of a poorer quality than either reversed plaques (total IgM globulin-producing cells) or IgM anti-BIG plaques. Therefore anti-MIG plaques can only be counted accurately at a low concentration of less than about 50 PFC per slide, and most of the counts of these plaques are probably underestimates and certainly relatively less than estimates for the other more clearly defined plaques.

Fig. 1 Photographs of reversed plaques (total IgM globulin) (*a*); direct plaques against TNP- (*b*); and indirect plaques against MIG (*c*) and BIG (*d*) 4 d after the intraperitoneal injection of 20 μg LPS (*Salmonella typhosa* 0901 B). Although the anti-MIG plaques have a less complete haemolysis than the other plaques, microscopic examinations of dried glutaraldehyde fixed slides, stained with Wright, shows that all plaques have at least one lymphoid cell within them and most have only one which is unequivocally at the centre: this observation is facilitated by the very high frequency of anti-MIG PFC in the spleens of LPS injected mice.



After the injection of LPS into CBA mice there is a dramatic rise in PFC directed against a variety of antigens[1]. In the context of the reversed plaques and the four target antigens used in the experiments described here, there are two distinct types of IgM—direct and indirect. The direct types are IgM antibodies specific for TNP- and for sheep red blood cells (SRBC) which appear on addition of guinea pig complement but do not require the presence of an anti-μ developing serum and the indirect types are anti-BIG, anti-MIG and reversed plaques which, in addition to a source of complement, require a rabbit anti-(mouse)-μ developing serum. In Fig. 2 it can be seen that the peak of the direct IgM response after LPS injection is on day 3 and for indirect IgM it is on day 4. It is not impossible that these operationally distinct types of IgM may reflect the presence of two sub-classes of murine IgM related in some, as yet undefined, way to the two sub-classes described by Plotz *et al.*[12].

The results shown in Fig. 3 show that anti-MIG plaques can be inhibited by free MIG at lower than the concentrations of free BIG previously reported for the equivalent LPS stimulated anti-BIG plaques[1]. Perhaps a better comparison for the inhibition of anti-MIG plaques are the higher avidity plaques (inhibited by less free BIG) reported after immunisation with BIG+LPS: essentially these are PFC arising after immunisation with MIG+LPS. Figure 3 also shows that free BIG does not inhibit anti-MIG plaques, so it seems likely from this and the previously published data, that anti-BIG and anti-MIG-PFC are different populations of cells. The much greater inhibition of anti-MIG PFC by the Fc fragment of mouse IgG than by the Fab fragment, is consistent with those plaques being the result of the secretion of a rheumatoid factor-like molecule[14,15].

The reversed plaque assay used here allows an estimate to be made of the total number of cells synthesising IgM globulin. It is an excellent method for measuring the effectiveness of a very wide range of mitogens and furthermore it makes it possible to express both mitogen and immunogen stimulated responses as—that proportion of the elevated total IgM globulin production which is specific for a particular target antigen. Some recent experiments (unpublished data and ref. 16) with mice immunised with SRBC show that (1) less than 20% of the IgM-producing cells, which appear 4 d after antigen injection, can be assigned as having anti-SRBC specificity and (2) a large proportion of these IgM-producing cells are anti-MIG PFC: the degree of overlap, if any, between anti-SRBC and anti-MIG plaques is not known. Although there is no formal proof that anti-MIG specificity is due to a conventional antibody site, Metzger has argued convincingly that human M-anti-G specificity is due to such a conventional antibody activity[17].

The existence of large numbers of auto-antibody-forming cells arising as a result of exposure to LPS or immunogens, in the presence of auto-antigen, suggests that this ability to manifest auto-immunity has arisen not as an accident, but as the consequence of particular selective pressures. The response directed against hidden auto-erythrocyte antigens or altered-self proteins may be a mechanism for the opsonisation of damaged or aged red blood cells and protein molecules, which assists their removal by macrophages from the blood[18]. Protection of cells against osmotic and mechanical stress has also been suggested as a function for auto-antibodies[19]. The possible role of an IgM response to bound or altered IgG (antibody) as a first step in the complement cascade has been proposed[1]. As an extension of the hypothesis that auto-immunity can be interpreted as a break of tolerance[20] it has been suggested that large numbers of auto-reactive B-cells arise as the result of the elimination of specific regulatory T cells[21–23].

Since there is such a large capacity for synthesising and secreting IgM molecules which bind IgG (Fc) it is tempting to propose these molecules as candidates for a cybernetic network. However, the relative inability of Fab to inhibit

anti-MIG plaques (Fig. 3) makes it unlikely that this hypothetical network could have the fine specificity necessary for Jerne's proposed idiotype-dependent network[24]. Another possibility may lie in a role for auto-antigens in addition to environmental hetero-antigens, driving a mechanism for generating immune competence in B cells (immunological repertoire) in a manner analogous to that proposed for the role of MHC in driving the generation of diversity in T cells[25].

As stated above, the injection of an immunogen (SRBC) leads to an increase in total IgM globulin production, of which only a minor proportion can be assigned as having anti-SRBC specificity. If this is a general result of the injection of an immunogen and preliminary experiments suggest that this may well be so, then it could be imagined that the main difference between a mitogen and an immunogen is that the former has a random effect in binding to any and all cells, whereas the immunogen is concentrated on those lymphocytes with receptors for antigen: these may be IgD in virgin precursor cells and Ig of the class eventually secreted in memory cells[15]. This mechanism is superficially similar to the hypothetical mechanism proposed by Möller[5], who suggests that the role of antigen-receptors is to concentrate antigen (mitogen) near the mitogen-receptor which triggers the lymphocyte to differentiate into a cell secreting IgM. However, it is unlikely that this is a general mechanism since completely separate, nonimmunogenic antigen and adjuvant molecules can successfully trigger an IgG response[26].

I thank Dr G. G. B. Klaus for a critical reading of the manuscript.

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Received 23 February; accepted 29 April 1978.

1. Dresser, D. W. & Popham, A. M. *Nature* **264**, 552–554 (1976).
2. Dresser, D. W. & Phillips, J. M. *Ciba Symp.* **18**, 3–28 (1973).
3. Primi, D., Hammarström, L., Smith, C. I. E. & Möller, G. *J. exp. Med.* **145**, 21–30 (1977).
4. Coutinho, A., Gronowicz, E., Bullock, W. W. & Möller, G. *J. exp. Med.* **139**, 74–92 (1974).
5. Möller, G. *Transplant Rev.* **23**, 126–137 (1975).
6. Primi, D., Smith, C. I. E., Hammarström, L. & Möller, G. *Cell. Immun.* **34**, 367–373 (1977).
7. Steele, E. J. & Cunningham, A. J. *Nature* **274**, 483–484 (1978).

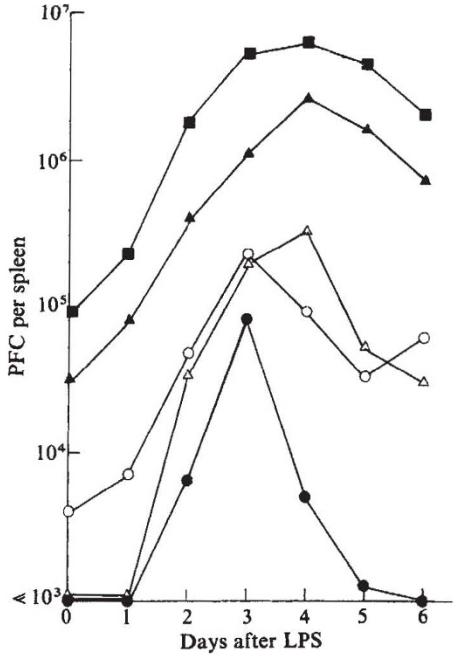

Fig. 2 The rise and fall in numbers of PFC after the intraperitoneaf injection of 20 μg LPS into male CBA/Ca mice aged 10–20 months (lesser responses are obtained in younger mice), bred in an SPF unit and maintained in minimal disease conditions during experimentation. ■, Reversed-plaques (total IgM globulin); ▲, anti-MIG; and △, are anti-BIG plaques; these three categories of plaque depend on the presence of a rabbit anti-(mouse) μ developing serum and consequently can be described as indirect μ plaques in contrast to direct μ plaques which do not require development. Two examples of direct plaques stimulated by LPS are included here, ○, anti-TNP and ●, anti-SRBC. The detailed methodology of Jerne's haemolytic plaque assay and of Molinaro and Dray's reversed plaque assay are described elsewhere[10] together with an account of the cold chromic chloride method for coupling proteins to target erythrocytes. MIG was prepared by precipitation from mouse serum in half saturated ammonium sulphate, which was followed by purification on a column of Sepharose-(staphylococcal) protein A (Pharmacia) when the MIG was required for inhibition purposes. All classes of mouse IgG bind well to protein A in 0.01 M Tris-HCl *p*H 8.1 buffer and can be eluted in a pure state as judged by immunoelectrophoresis, using buffer at *p*H 2.8. MIG for coupling to target erythrocytes is further fractionated by precipitations in 25% ethanol at *p*H 6.5 (Cohn fraction II)[11]. In addition to the titration of the rabbit anti-(mouse)-μ developing serum to find the optimal concentration, it was necessary to titrate the (MIG-Sepharose and SRBC absorbed) guinea pig complement against each batch of MIG-SRBC which was used: the optima lay between 1 and 5% in the present experiments. All lymphoid and target cells were kept in Hank's solution containing 0.5% Difco (Bacto) gelatine (HG). 'Day zero' mice did not receive LPS and these results can be considered as an indication of background levels.

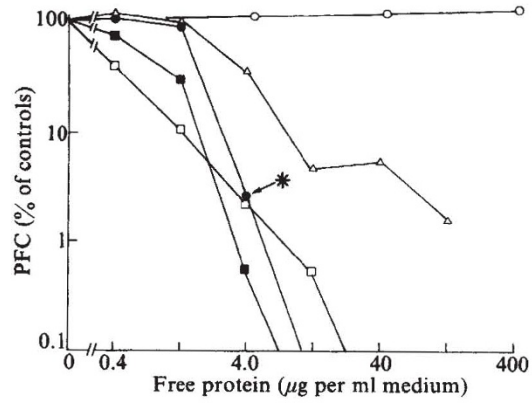

Fig. 3 The inhibition of day 4 post-LPS anti-MIG PFC by free proteins: MIG, ●; BIG, ○; mouse IgG-Fc, □; mouse IgG-Fab, △; and Fc+Fab, ■. The 100% values do not differ significantly from the day 4 values in Fig. 2. I thank G. G. B. Klaus for the Fc and Fab preparations, made by papain digestion and fractionation on Sepharose–protein A. Similar inhibition curves were obtained using Fc and Fab prepared by papain digestion and chromatography on DEAE–cellulose. MIG which had not been re-fractionated on Sepharose–protein A and which presumably contained traces of IgM, caused a general lysis of MIG-coated target cells. The observed degree of inhibition by Fab might be due to a small amount of contamination of the preparation by Fab or due to the exposure of normally hidden auto-antigenic Fab determinants similar to those described by Mandy *et al.* in the rabbit[13]. It is unlikely that the observed inhibition effect is due to the free-MIG interfering with the rabbit anti-(mouse)-μ developing serum, since a threefold increase in the concentration of developing serum (at the point marked *) has no effect on the number of detectable plaques. Passage of MIG down a column of Sepharose–(mouse)-IgM (TEPC 183) did not result in a significant reduction in plaque number when this material was used as a target antigen. In contrast, reversed plaques were totally ablated, if an IgG preparation of goat anti-(mouse)-μ was passed down a similar column before being coupled to target erythrocytes. This result argues against a possibility that 'anti-MIG' plaques are due to a significant amount of anti-μ activity in the MIG preparations used to coat the target SRBC.

8. Dresser, D. W., Keeler, K. D. & Phillips, J. M. *Biochem. Soc. Trans.* **4**, 34–38 (1976).
9. Taylor-Upsahl, M. M., Abrahamsen, T. G. & Natvig, J. B. *Clin. exp. Immun.* **28**, 197–203 (1977).
10. Dresser, D. W. in *Handbook of Experimental Immunology* (ed. Weir, D. M.) Ch. 28 (Blackwell, Oxford, 1978).
11. Cohn, E. J. *et al. J. Am. chem. Soc.* **68**, 459–475 (1946).
12. Plotz, P. H., Talal, N. & Asofsky, R. *J. Immun.* **100**, 744–751 (1968).
13. Mandy, W. J., Fudenberg, H. H. & Lewis, F. B. *J. Immun.* **95**, 501–509 (1966).
14. Johnson, P. M. & Page-Faulk, W. *Clin. Immun. Immunopath.* **6**, 414–430 (1976).
15. Elson, C. J., Naysmith, J. D. & Taylor, R. B. *Int. Rev. exp. path.* **19** (in the press).
16. Parkhouse, R. M. E. & Dresser, D. W. in *The Secretory Immune System and Caries Immunity* (eds McGhee, J. R. & Mestecky, J.) (Plenum, New York, 1978).
17. Metzger, H. in *Tolerance, Autoimmunity and Aging* (eds Sigel, M. M. & Good, R. H.) 120–126 (C. C. Thomas, Springfield, Illinois, 1972).
18. Kay, M. M. B. *Proc. nam. Acad. Sci. (U.S.A.)* **72**, 3521–3535 (1975).
19. *Najjar, V. A. Lymphology* **3**, 23–31 (1970).
20. Weigle, W. O. *Clin. exp. Immun.* **9**, 437–477 (1971).
21. Fudenberg, H. H. *Am. J. Med.* **51**, 295–298 (1971).
22. Gershon, R., Cohen, P., Hencin, R. & Liebhaber, S. *J. Immun.* **108**, 586–590 (1972).
23. Cunningham, A. J. *Nature* **254**, 143–144 (1975).
24. Jerne, N. K. *Ann. Immun. Inst. Pasteur* **125C**, 373–389 (1974).
25. Jerne, N. K. *Eur. J. Immun.* **1**, 1-9 (1971).
26. Dresser, D. W. *Br med. Bull.* **32**, 147–151 (1976).

High proportion of Ig-producing cells making autoantibody in normal mice

'NATURAL' serum antibodies are thought to arise as a result of stimulation by environmental antigens. Evidence suggests, however, that many such antibodies in normal healthy individuals are specific for and may be induced by a variety of self components. Many of these autoantibodies are directed against 'buried' or 'enzyme-revealed' self antigens and include those specific for mouse reproductive organs which crossreact with human group A erythrocytes[1], human and rabbit immunoglobulins treated with proteolytic enzymes[2,3], denatured DNA[4], enzyme-treated human lymphocytes and erythrocytes[5–8], and mouse erythrocytes treated with the proteolytic enzyme bromelain[9]. This widespread occurrence of autoantibodies could suggest that the normal immune system is preoccupied in reacting against self antigens. One way of measuring the extent of this reactivity is to compare the number of B cells making a given autoantibody(s) with the total number of B cells producing immunoglobulin (Ig) irrespective of specificity. We have previously shown that normal conventional and germ-free mice possess in their spleens considerable numbers of PFC against bromelain-treated isologous mouse erythrocytes (BrM), an 'internal' antigen of mouse erythrocytes[9,10]. We have measured the proportion of Ig-secreting cells in the lymphoid organs of normal CBA/H mice forming PFC against BrM and found that in some (but not all) major lymphoid organs between 1% and >50% of the existing or potential Ig-secreting cells are specific for BrM.

All PFC assays were carried out using the liquid monolayer system of Cunningham and Szenberg[11]. Bromelain-treated mouse erythrocytes were used to detect BrM PFC (ref. 10). Ig-secreting cells (Ig PFC) were detected by the reverse plaque method[12] using sheep erythrocytes coated with the globulin fraction of a polyvalent hyperimmune sheep anti-mouse Ig serum by the chromium chloride method[13]. Reverse plaques require the presence of a rabbit anti-mouse Ig-developing serum at about the same concentration as used to detect conventional specific indirect plaques (see ref. 14). The detection system is polyvalent, revealing B cells producing IgM, IgG and IgA (data not shown). The direct BrM PFC are formed by cells making IgM antibody. Thus, the 'true' proportion of BrM PFC to total IgM PFC will be greater than the frequencies reported here if significant numbers of cells secreting Ig classes other than IgM are present in a given organ.

The numbers of BrM PFC and Ig PFC in various organs of CBA/H mice are shown in Tables 1 and 2. Table 1 displays the 'natural' antibody producers in freshly collected cell suspensions, whereas Table 2 shows results for cells cultured for several days in the presence of lipopolysaccharide (LPS)—a polyclonal B cell activator[15]—to identify 'potential' antibody producers.

The spleens of normal male or female CBA/H mice contain about 5,700 Ig PFC per 10^6 cells (Table 1). Ig PFC are also found in other lymphoid organs, notably mesenteric lymph nodes ($3{,}000/10^6$), bone marrow ($1{,}030/10^6$), thymus ($310/10^6$) and Peyer's patches ($15{,}000/10^6$). Few are present in popliteal and parathymic lymph nodes or in freshly collected peritoneal or pleural cavity cells. BrM PFC have a different distribution. Expressed as a percentage of Ig PFC, BrM PFC are high in spleen (1.6%) and bone marrow (7.4%), low in thymus (0.6%) and mesenteric lymph nodes (0.2%) and undetectable in Peyer's patches (< 0.01%).

If peritoneal cells are cultured *in vitro* they can be shown to rapidly produce many BrM PFC (refs 16 and 17). It has been shown that the appearance of BrM PFC in peritoneal (and spleen) cell cultures requires little cell division but does depend on RNA and protein synthesis[16]. The mechanism of the normal *in situ* suppression of these potential PFC is not clear, although there is some evidence that an antigen dependent blockading process is involved[18].

The large number of BrM PFC generated in short-term peritoneal cell cultures suggested that much of the Ig-secreting cell potential in this site may be directed against BrM. Table 2 summarises results of a series of experiments comparing the proportions of BrM and Ig PFC after short-term culture in the presence of LPS. For spleen 1–2% of Ig PFC were BrM specific (same as before culture, Table 1). Peyer's patch cells, containing few BrM PFC before culture (Table 1), also show very low numbers after culture. Parathymic lymph nodes behave similarly in this respect (data not shown). The most striking result occurs in cultures of peritoneal or pleural cavity cells, where between 40% and 95% of the Ig PFC are BrM specific. It is noted that older mice, particularly retired breeder females contain more potential PFC than younger mice. Lord and Dutton[16] have reported no increase with age in the number of BrM PFC generated by peritoneal cells *in vitro*. Our findings agree, however, with earlier studies by Nossal *et al.*[19] who showed that peritoneal cell cultures from retired breeder females produce many more PFC against sheep erythrocytes—an antigen known to crossreact with BrM[17,18]—than young male mice.

In other experiments (data not shown) we have shown that removing cells forming rosettes with BrM (1% in spleen, 20%

Table 1 Tissue distribution of Ig-secreting cells and BrM PFC in CBA/H mice*

	PFC per 10^6 cells		
	Ig	BrM	%BrM†
Spleen	5,700 ± 350‡	87 ± 17‡	1.6 ± 0.4‡
Thymus	310 ± 150	1.4 ± 1.1	0.6 ± 0.2
Parathymic lymph nodes	180 ± 48	5 ± 3	2.2 ± 1.2
Mesenteric lymph nodes	3,000 ± 830	7 ± 4	0.2 ± 0.1
Popliteal lymph nodes	38 ± 21	≤ 14	
Bone marrow	1,030 ± 370	70 ± 22	7.4 ± 0.7
Peyer's patches	15,000 ± 3,400	< 1	< 0.01
Peritoneal cells	250 ± 190	42 ± 24	15 ± 11
Pleural cavity cells	< 50	< 50	

* Results summarised from four experiments using normal male (two experiments) and female (two experiments) CBA/H mice (age range 1.5–5 months). In each experiment pooled tissues from four age- and sex-matched mice were used. Viable nucleated cells were determined by trypan blue exclusion. The average viable cell counts per organ ± s.e.m. ($\times 10^{-6}$) were: spleen (62 ± 11), thymus (129 ± 40), parathymic lymph nodes (4.5 ± 0.8), mesenteric lymph nodes (13 ± 0.6), popliteal lymph nodes (0.9 ± 0.25), bone marrow (16 ± 2.3, per tibia and femur), Peyer's patches (0.9 ± 0.2), peritoneal cells (2.6 ± 0.5), and pleural cavity cells (1.1 ± 0.1).
† %BrM = (BrM PFC/Ig PFC) × 100.
‡ Arithmetic mean ± s.e.m. for four experiments.

0028–0836/78/0274—0483$01.00