Table 2 Forward mutation rates induced in prokaryotic organisms

Organism	Genome size (daltons)	Radiation		EMS		References
		Observed values	Extrapolated values	Observed values	Extrapolated values	
E. coli	2.8×10^{9}*	1×10^{-9}†	6.74×10^{-10}	2.5×10^{-4}	6.75×10^{-5}	*8, †9
T_2	1.2×10^{8}*	7.8×10^{-13}†	3.2×10^{-11}	7×10^{-3}‡	1.8×10^{-6}	*22, †23, ‡24
T_4	1.5×10^{8}*	1.08×10^{-8}†	4.5×10^{-11}	–	3×10^{-6}	*22, †25
φX174 and S13	1.7×10^{8}*	–	7.91×10^{-13}	2.91×10^{-16}†	2.38×10^{-8}	*26, †27

Asterisks, daggers and double daggers apply as in Table 1.

explanation would be to measure mutation rates in conditions such that only changes in the structural gene are detected in organisms of different genome size. Another prediction from these models is that the mutation rate will be determined mostly by the size of the structural gene when the proportion of controlling DNA is small. Thus one would expect that organisms with most of their genomes consisting of structural genes would have similar mutation rates, presumably much like that of *E. coli*. Although we have not been able to find much data on this matter, Table 2 shows that neither this prediction nor an extrapolation of the ABCW relation seems satisfactory.

The correlations, regardless of the nature of the mechanism underlying them, provide support for the proposal to express the exposure to chemical mutagens as an equivalent radiation dose. One of the major difficulties in the regulation of human exposure to mutagens is the quantitative estimation of the genetic risk. The most thorough assessment of mutagenicity has been carried out for ionising radiation and maximum population exposure limits have been laid down. On this basis a unit which could be used to quantify human exposure has been suggested, the rem-equivalent-chemical (rec or "radequiv.") which is defined as that concentration of a chemical mutagen that produces an amount of genetic damage equal to that produced by 1 rem of chronic irradiation[6,7]. For such a unit to be meaningful the ratio of mutagenicity of the chemical to radiation must be the same for man and the test organisms. The curves in Figs 1 and 2 indicate that there is a remarkably constant relationship over several orders of magnitude of mutation rate, although there is some uncertainty in the data for any one organism. Since the lines of best fit have somewhat different slopes (1.1 and 0.9) we have used the ratio of the fitted values at the geometric mean (*Arabidopsis*) to calculate the rec value of 4×10^{-6} M. Expressed in terms of rads, therefore, treatment by a 1-M solution is equivalent to about 2.5×10^{5} rad.

There are, of course, other problems in estimating the human risk and there is the problem of assessing the benefit of the chemical also. Nevertheless, if similar correlations are found for other mutagens, rough extrapolations from the simple, rapid microbiological screens to human risk can be made to provide a quantitative evaluation. This would be useful in planning mammalian experiments and evaluating the results. Mammalian systems will still be important because of the potential influence of uniquely mammalian aspects of metabolism, absorption, and excretion of compounds and the possibility of classes of damage that do not occur in prokaryotes, such as chromosome breakage and non-disjunction. Nevertheless, the rec concept has many possible uses in the light of the data presented here which suggest that there is a relatively constant value of rec for all organisms.

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Stimulation of mouse lymphocytes by insoluble anti-mouse immunoglobulin

IT is generally agreed that B-cell surface immunoglobulin (Ig) functions as the receptor for antigen, and so accounts for the specificity of the immune response by clonal selection. It is not known, however, how the combination of surface Ig with antigen stimulates the cell to proliferation and/or differentiation to secrete antibody at a high rate[1]. A signal could be delivered directly through the receptors, either by an allosteric transition in the receptor itself or by cross-linkage or aggregation of receptor Ig in the fluid cell membrane, analogous to antigen-induced or anti-IgE-induced histamine release from mast cells[2]. Alternatively, the receptor may act passively as an antigen-specific 'address' for the delivery of an antigen-associated non-specific (that is, potentially polyclonal) signal to some other site on the cell surface[3].

To test whether optimal cross linkage of receptor Ig can stimulate B cells, I have coupled purified rabbit anti-mouse Ig (predominantly anti-κ specificity) covalently to the surface of polyacrylamide beads. The anti-Ig beads were added to normal mouse spleen cell cultures. As Fig. 1 (solid bars) and Table 1 show, anti-Ig beads are strong polyclonal mitogens for normal mouse spleen cells; they stimulate a 10–20-fold increase in incorporation of ^{125}I-iododeoxyuridine (IdUrd) at 48 and 72 h. Control beads prepared with purified rabbit anti-*p*-azobenzenearsonate (anti-ars) antibodies had no effect (Fig. 1 and Table 1). Isotope incorporation data were confirmed by direct microscopic inspection: the number and percentage of blast cells in anti-Ig bead-stimulated cultures were ten times greater than in controls. The blasts were B cells as shown by positive immunofluorescent staining for surface Ig, while parallel phytohaemagglutinin-stimulated cultures yielded surface Ig negative blasts.

This is the first report of significant stimulation of mouse B lymphocytes by purified anti-mouse Ig. Since soluble anti-Ig

stimulates rabbit[8], pig[9] and chicken[10] lymphocytes, the failure of soluble anti-Ig to stimulate lymphocytes of other species remains a puzzle[1]. Insolubilisation on a surface has been shown to convert two other mouse B-cell ligands, which in soluble form are mitogens for T cells only—concanavalin A[11] and phytohaemagglutinin[12]—into B-cell mitogens, and to convert haptens or thymus dependent antigens into thymus independent antigens[13]. Insolubilisation on a surface creates a matrix of ligands at a fixed density, prevents internalisation and affects redistribution on the cell surface (although it does not prevent capping of receptors and release from the surface[14]). Which of these effects is crucial in this system remains to be determined.

A high density of anti-Ig on the bead surface was required for stimulation (Table 1). The peak response occurred with 64 and 130 μg of antibody per ml of beads. A concentration of 400 μg ml^{-1} stimulated suboptimally, whereas 6 μg ml^{-1} and 1 μg ml^{-1} failed to stimulate. Five times fewer beads per culture gave half the response, without a change in the optimal density per bead (data not shown). Since the beads (BioGel P30) exclude Ig from the interior, all the anti-Ig was at or near the bead surface. Beads are nearly close packed at 400 μgm l^{-1} if one assumes the limit of a smooth spherical surface of radius 50 μm. This rather sharp dependence on ligand density is expected if the receptor Ig signal to the interior of the cell depends on the formation of small local aggregates of receptors, as seems to be the case with mast cell triggering[15]. To induce receptor aggregates on the cell surface, the anti-Ig molecules must be close together on the semi-rigid bead surface, since the density of adjacent pairs (or trios) of anti-Ig molecules would vary nearly as the square (or cube) of the anti-Ig density, if the distribution of anti-Ig on the bead surface is random.

Taken together with the extensive studies of the effect of density of antigenic determinants and molecular structure on the immunogenicity of thymus-independent antigens[16], these results support the view that one route to B-cell activation is a critical extent and duration of cross linkage of cell surface

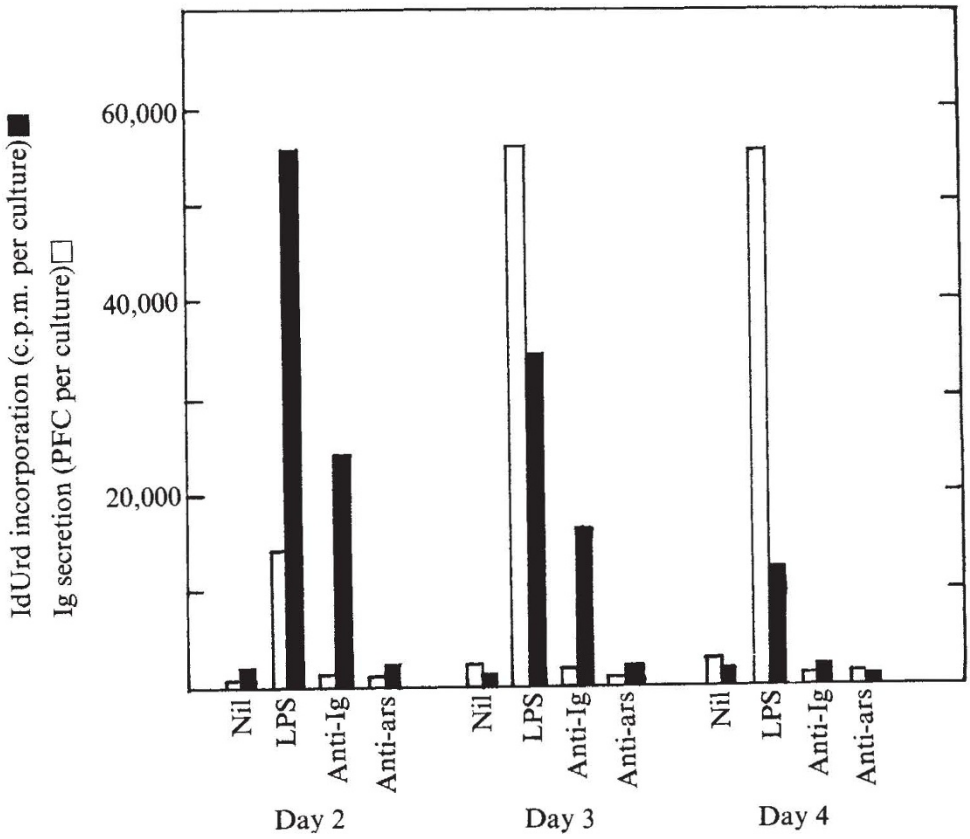

Fig. 1 Comparison of proliferation (solid bars, ^{125}IdUrd incorporated) and number of Ig-secreting cells (open bars, PFC) in normal spleen cell cultures stimulated with lipopolysaccharide (LPS, from *E. coli* 055:B5, Difco, 100 μg ml^{-1}) or antibody-coupled polyacrylamide beads (130 μg ml^{-1} settled beads; 5×10^3 beads per culture). Values shown are the arithmetic means of triplicate cultures. Standard errors were of the same order as those shown in Table 1. Normal 8–12-week CBA mouse spleen cells were washed with medium and cultured at 5×10^5 cells per 200 μl of modified Eagle's medium containing extra glutamine, antibiotics and 10% heat inactivated foetal calf serum in flat bottom wells in micoplates (Linbro IS-FB-96) in an atmosphere of 10% CO_2 in air. 1 μCi ^{125}IdUrd (100 μCi μg^{-1}, Radiochemical Centre, Amersham), was added to some cultures 4 h before collection on to glass fibre filters. Parallel cultures were collected with a Pasteur pipette, washed twice, and assayed for PFC using the plaque assay described below. The anti-mouse Ig antibodies were isolated from pooled hyperimmune antisera to normal mouse Ig and to a γ2a,κ and a γl,κ myleoma protein, using a Sepharose immunoadsorbant, and eluted with glycine-NaCl buffer, *p*H 2.4. The immunoadsorbant (loaned by D. Kilburn) was coupled with a mouse κ chain dimer isolated from the ascites fluid of mice bearing MOPC 70A plasmacytoma. Hyperimmune anti-ars antisera were raised in rabbits to a haemocyanin azo conjugate, and the antibodies were purified on arsanilate Sepharose. The purified anti-Ig antibodies were trace labelled with ^{125}I by a modification of the Chloramine T technique[4]. Antibody was coupled to polyacrylamide beads (BioGel P30, 100–200 mesh, BioRad) by the acyl azide procedure of Inman and Dintzis[5]. The hydrazide intermediate (0.75 mmolg^{-1}) was reacted with 0.15 M $NaNO_2$ in 0.3 M HCl for 3 min. After washing as described, the beads were added to an equal volume of antibody in 0.09 M sodium tetraborate, *p*H 9, and tumbled slowly overnight at 4 °C. Unreacted azide groups were reconconverted to the amide. The beads were washed extensively with 6% saline *p*H 8.6, followed by sterile phosphate-buffered saline, *p*H 7.2, in which they were stored at 4 °C with azide. Ten per cent of the antibody added at 1 mg ml^{-1} beads was coupled to the beads by this procedure. Before use they were washed extensively with sterile saline and then culture medium. Immunoglobulin-secreting cells were counted using an adaptation of the reverse haemolytic plaque assay developed by Molinaro[6]. Sheep red cells were coated with anti-mouse Ig (anti-κ) using hybrid antibody[6], and used in a monolayer plaque technique[7]. Normal spleen contained 4,000–10,000 direct PFC per 10^6 nucleated cells. In LPS-stimulated cultures, about 20% of the cells were PFC at days 3 and 4. In general, anti-Ig coated red cells gave 1,000 times the number of direct (anti-sheep red cell) plaques obtained with uncoated red cells.

Table 1 Effect of surface density of anti-Ig on stimulation

		^{125}IdUrd incorporated (c.p.m. per culture ± s.e.)	Ig-secreting cells (PFC per culture ± s.e.)
Nil		1,190±50	3,480±260
LPS (100 μg ml^{-1})		17,300±540	63,300±2,400
Unmodified beads		1,200±80	1,690±640
Anti-Ig beads	1 μg ml^{-1}*	1,070±30	3,480±140
	6 μg ml^{-1}	1,254±30	1,720±290
	64 μg ml^{-1}	22,180±1,310	5,690±350
	64 μg ml + LPS	17,970±2,110	63,900±13,140
Nil		1,350±30	2,270±180
LPS (100 μg ml^{-1})		34,870±1,350	56,130±1,540
Anti-Ig beads	64 μg ml^{-1}	15,040±1,250	1,030±30
	130 μg ml^{-1}	16,730±2,390	1,450±130
	400 μg ml^{-1}	5,450±770	1,120±280
Anti-ars beads	400 μg ml^{-1}	634±15	1,660±280

Culture conditions were the same as Fig. 1. Cultures were collected on day 3. Arithmetic means ± the standard error of the mean for triplicate cultures are shown.
*μg of purified antibody per ml of settled beads (10^6 beads per ml). 5×10^3 beads, enough to form a layer two beads thick on the bottom of the well, were added to each culture.

structures, one of which is receptor Ig. But two alternative explanations must be considered. The first is that the anti-Ig beads stimulate B cells indirectly, by stimulating a surface Ig-bearing, non-B cell (macrophage or activated T cell) to release a B-cell activating factor. The second is that the Fc region of the surface-bound anti-Ig delivers an activating signal to the B cell through its Fc receptors. This is unlikely since antigen–antibody complexes[17] and beads densely coupled with rabbit anti-hapten antibody (Table 1) do not stimulate mouse spleen cells. These alternatives are being tested using purified B cells and beads conjugated with the $(Fab')_2$ fragment[18] of anti-Ig. The polyacrylamide bead itself is unlikely to interact with any cell surface structure or deliver any signals to the cell. First, it is the substrate of choice for cellular immunoadsorbants since, unlike Sephadex, glass beads or other plastics, it does not adsorb sticky cells nonspecifically, as shown by 99±1% yields of passed cells in column fractionation procedures[19]. Second, it is not a polyclonal activator as shown by normal requirements for antigen and accessory cells in the antibody response to sheep erythrocytes of mouse spleen cells cultured directly on a polyacrylamide surface in a microwell technique[20]. Third, unmodified polyacrylamide beads or beads coated with rabbit anti-hapten antibodies failed to stimulate cells in the experiments reported here and elsewhere[13]. Sephadex G-15 beads coupled[21] with anti-Ig at 30 μg ml^{-1} and 170 μg ml^{-1} failed to stimulate, although quantitation was difficult because the beads bound considerable ^{125}IdUrd in the absence of cells. The lack of stimulation may have been due to the wrong surface density of anti-Ig or to interaction of the Sephadex with the cell surface.

Although the anti-Ig beads were comparable with LPS in their ability to stimulate cell proliferation, it was striking that unlike LPS or other B-cell mitogens, with the exception of dextran sulphate[22], they failed to stimulate significant increases in numbers of immunoglobulin-secreting cells (Fig. 1 and Table 1). Since the reverse haemolytic plaque assay (see Fig. 1 legend) might be relatively inefficient for IgG and IgA-secreting cells, the blasts in bead-stimulated cultures were examined for intracellular Ig using a polyvalent fluorescent anti-Ig antiserum, and found to be negative. This result has several possible interpretations: (1) The anti-Ig beads may be turning off Ig synthesis in the stimulated cells. I have found that free anti-Ig inhibits plaque-forming cells without much effect on IdUrd incorporation in LPS-stimulated cultures (data not shown) in agreement with Andersson *et al.*[23] who pretreated cells with anti-Ig at 4 °C before culturing with LPS. The anti-Ig beads, however, did not turn off Ig secretion in cultures stimulated with LPS (Table 1). (2) T cells or macrophages may be inhibiting Ig secretion. (3) The anti-Ig beads may be giving a proliferation signal to a subpopulation[24] of B cells which requires a second differentiation signal to proceed to a high rate of Ig synthesis and secretion[25,26]. Soluble anti-Ig stimulates proliferation of rabbit lymphocytes by cross linking receptors, but antibody secretion depends on the addition of T-cell factors[27]. This radical dissociation of proliferation from immunoglobulin secretion in anti-Ig bead-stimulated B cells will prove useful as a model for regulation of antibody synthesis in antigen-activated B cells in the mouse, where the dissection of interacting components in the immune response has proceeded much further than in other species.

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Identical sequence of light chains from rabbit anti-streptococcal antibodies

CONSIDERABLE advances have been made with special breeding programmes in the selection of rabbits with amplified immune responses to polysaccharides specific for different streptococcal groups[1,2]. Levels of group specific antibodies of 50 mg ml^{-1}