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# Opposing effects of *xid* and *nu* mutations on proliferative and polyclonal antibody and autoantibody responses to peptidoglycan, LPS, protein A and PWM

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**Summary.** We have compared the *in vitro* and *in vivo* mitogenic and polyclonal antibody (IgM-, IgG-, IgA- and anti-SRBC-secreting PFC) and autoantibody (IgM anti-ssDNA and anti-bromelin-treated mouse RBC-secreting PFC) responses to peptidoglycan (PG), LPS, protein A and PWM in homozygous *xid* or *nu* and normal mice. Our results demonstrated opposing effects of *xid* and *nu* on polyclonal B cell activation; in general, *xid* retarded and *nu* enhanced or did not change these responses. These effects, however, were greatly dependent on the *in vitro* or *in vivo* conditions of the stimulation and the type of polyclonal activator used and antibody assayed (isotype and specificity). *In vitro*, in *xid* mice, the numbers of all PFC assayed and proliferative responses were lower than in normal mice, whereas in nude mice the numbers of PFC were mostly unchanged, and proliferative responses were increased (PG, LPS) or decreased (protein A, PWM). The *in vitro* frequencies of autoantibody-secreting cells were similar (anti-DNA) in *xid*, nude and normal mice, or lower (anti-RBC) than normal in *xid* mice. *In vivo*, unstimulated *xid* mice had lower than normal numbers of IgM-, IgG- and autoantibody-secreting cells and higher numbers of IgA PFC, but in stimulated *xid* mice, the numbers of all Ig PFC were similar to normal, whereas anti-DNA and anti-RBC PFC were still depressed. The frequencies of anti-DNA and anti-RBC PFC were also lower than normal in *xid* mice *in vivo*. Nude mice *in vivo* had higher than normal numbers and frequencies of anti-DNA PFC and lower numbers of IgM and anti-SRBC PFC. These results indicate preferential retardation of autoantibody-secreting cells in *xid* mice *in vivo* and preferential enhancement of these cells in nude mice *in vivo*. Since in *xid* mice *in vitro* PG- and LPS-induced responses were similarly diminished, PG, like LPS, appears to primarily activate a late-maturing B cell subpopulation affected by the *xid* mutation.

Abbreviations: Con A, concanavalin A; HB MRBC, bromelin-treated mouse red blood cells; Ig, immunoglobulin(s); LPS, lipopolysaccharide; PFC, plaque forming cell(s); PG, peptidoglycan; PHA, phytohaemagglutinin; PWM, pokeweed mitogen; SRBC, sheep red blood cells; ss, single-stranded.

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## INTRODUCTION

Immunologically deficient mutant strains of mice, such as athymic nude (*nu/nu*) and CBA/N (homozygous or hemizygous for the *xid* mutation on the X chromosome and lacking Lyb $3^+$, $5^+$, $7^+$ B cells), are very useful in studies on normal development and function of the immune system, on interactions of exogenous factors (such as microbial products) with the immune system, and on the immunopathological mechanisms of diseases that affect the immune system or result from its malfunction, e.g. autoimmune diseases. To dissect immunological mechanisms lead-

ing to autoimmune diseases, autoimmune responses have been studied in immunodeficient mice, or immunodeficiency genes have been introduced to murine strains that spontaneously develop autoimmune diseases. CBA/N-*xid*/*xid* mice, in contrast to normal (+/*xid* or +/+) mice, fail to make any significant numbers of cells secreting anti-bromelin-treated mouse red cell (HB MRBC) antibodies in response to *in vivo* stimulation with lipopolysaccharide (LPS) or malarial infection (Kemp *et al.*, 1980; Rosenberg, 1979). Introduction of the *xid* gene into autoimmune NZB, NZB × NZW $F_1$, BXSB or MRL-*lpr*/*lpr* mice ameliorates or greatly diminishes the symptoms of autoimmune disease (Kemp *et al.*, 1982; Ohsugi *et al.*, 1982; Smith, Chused & Steinberg, 1983; Steinberg *et al.*, 1982, 1983; Taurog *et al.*, 1981). Effects of neonatal thymectomy or the *nu* gene on induction of autoimmunity in otherwise normal mice are controversial. Some studies show no effect of the *nu*/*nu* genotype or thymectomy on induction of autoantibodies (Levy, Fridman & Neauport-Sautes, 1982; Primi, Smith & Hammarstrom, 1978), while others (Smith *et al.*, 1983b) demonstrated that thymectomy greatly predisposes mice to the development of autoimmunity induced by polyclonal B cell activators. Introduction of the *nu* gene into mouse strains which spontaneously develop autoimmune diseases (or neonatal thymectomy in these mice) results in the enhancement of autoimmunity and polyclonal B cell activation in NZB, NZB × NZW $F_1$ and BXSB mice (Milich & Gershwin, 1980; Smith *et al.*, 1983a) and retardation of the autoimmune disease in MRL-*lpr*/*lpr* mice (Theofilopoulos *et al.*, 1981). It is not known, however, if this diminution or enhancement of autoantibody production by the *xid* or *nu* genes is due to a total depression or enhancement of all polyclonal antibody responses, and if induction of different autoantibodies is similarly affected. Furthermore, it is also not known which polyclonal activators under what conditions could overcome this depression or induce further stimulation.

This study was undertaken to determine if the polyclonal activation of autoantibody-secreting cells was selectively affected by the *xid* and *nu* genes, or if these genes had the same effects on polyclonal activation of all IgM-, IgG- and IgA-secreting cells and the cells secreting autoantibodies and heteroantibodies in otherwise normal mice. We also sought to determine what effects the *xid* and *nu* genes have (in otherwise normal mice) on the frequency of autoantibody- and heteroantibody-secreting cells in unstimulated cells or cells polyclonally activated under different conditions (*in vitro* or *in vivo*) with different polyclonal activators. All these factors are of primary importance for the outcome of polyclonal activation in terms of the numbers and frequencies of cells secreting all Ig and autoantibodies. As polyclonal activators, in addition to LPS (which is the standard polyclonal B cell activator most often used in mice), we have selected three other polyclonal activators: peptidoglycan (PG), protein A and pokeweed mitogen (PWM), because of their greater potential application in the studies on polyclonal activation of both mouse and human lymphocytes (discussed in Dziarski, 1982a).

## MATERIALS AND METHODS

### *Mice*

Female, 5–8-week-old BALB/c (+/+), CBA/H (+/+) and CBA/N (*xid*/*xid*) mice from Dominion Laboratories (Dublin, VA), and athymic nude BALB/c (*nu*/*nu*) mice from Sprague Dawley (Madison, WI), fed standard Purina Formulab chow and water *ad libitum*, were used. Nude mice were kept in sterile laminar flow chambers and were given sterile food and water.

### *Polyclonal activators and mitogens*

Peptidoglycan (PG) was isolated from *Staphylococcus aureus* 845 cell wall by trichloroacetic acid extraction, its chemical composition and purity were established by paper chromatography, and contamination with exogenous endotoxins was ruled out (Dziarski & Dziarski, 1979). In addition, quantitative analysis of PG composition on an amino acid analyser, kindly performed by Dr Theodore A. Hare from the Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA, revealed the presence of the following amino acids (nmol/mg): gly (5744), ala (2554), lys (1716), glu (970), ile (135), ser (64), asp (50), thr (21) and leu (15). Other amino acids were not detected. This indicates the lack of any significant contamination of the PG preparation with proteins, since 97·5% of all amino acids in our PG were amino acids typical of *S. aureus* PG (Schleifer & Kandler, 1972). LPS, prepared from *Escherichia coli* 0128:B8 by a phenol-water extraction method, was obtained from Sigma Chemical Co. (St Louis, MO), protein A from *S. aureus* from Pharmacia Fine Chemicals (Piscataway, NJ) and PWM from GIBCO Laboratories (Grand Island, NY).

*Cell cultures*

Spleen cell suspensions and cultures were prepared as previously described (Dziarski, 1980; Dziarski, Dziarski & Levinson, 1980). For each *in vitro* experiment, spleen cells from at least three mice were pooled. For polyclonal activation, replicate (at least triplicate) and for mitogenicity, quadruplicate, cultures were established. The following, previously determined (Dziarski, 1980; Dziarski, 1982c; Dziarski & Dziarski, 1979; Dziarski *et al.*, 1980) optimal concentrations of mitogens or polyclonal activators were added at the initiation of the cultures: PG, 400 μg/ml; LPS, 50 μg/ml (polyclonal activation) or 100 μg/ml (mitogenicity); protein A, 1000 μg/ml; PWM, 1:100 (vol:vol, final dilution); PHA, 38 μg/ml; Con A, 3 μg/ml. Cultures were incubated for four days (polyclonal activation) or 48 hr (mitogenicity), which were the optimal periods for induction of Ig-secreting cells or proliferative responses, respectively.

In vivo *experiments*

Five to seven mice per group were injected i.v. with the predetermined (in previous (Dziarski, 1982b) and preliminary experiments) optimal dose for *in vivo* polyclonal activation of PG (40 mg/kg) or LPS (10 mg/kg). Control mice were left untreated. Four days after an injection, which was the optimal time for the *in vivo* polyclonal activation of all Ig- and autoantibody-secreting cells (Dziarski, 1982b), spleens were removed, single cell suspensions prepared in basal medium Eagle and plaque assays performed. Numbers of viable cells were counted using the trypan blue dye exclusion method.

*Plaque forming cell (PFC) assays*

The numbers of IgM-, IgG- and IgA-secreting PFC were determined as described (Dziarski, 1980) using the protein A haemolytic plaque assay with the same monospecific rabbit anti-mouse heavy chain developing sera (anti-IgM, pooled anti-IgG1 and anti-IgG2a, and anti-IgA). The numbers of cells secreting anti-single stranded (ss) DNA antibodies were determined as described (Dziarski, 1982a) with single stranded (heat-denatured) DNA bound to SRBC by $CrCl_3$. To visualize plaques, rabbit anti-mouse IgM developing serum (the same as in the protein A PFC assay) was used. Anti-mouse and sheep erythrocyte PFC assays were performed as described (Dziarski, 1982a) with bromelin-treated mouse RBC (HB MRBC, assay for anti-HB antigen autoantibodies) or untreated SRBC. Developing sera were not used in these assays because they did not increase the numbers of PFC. The results are expressed as PFC per number of viable recovered cells.

*Mitogenicity assay*

Incorporation of [$^3$H] thymidine into DNA was measured as described (Dziarski *et al.*, 1980). The results (incorporation of radioactivity into DNA) are expressed as counts per minute (c.p.m.) per culture.

*Statistical methods*

Student's *t*-test was used to determine the significance of the observed differences in the numbers of antibody secreting cells or the proliferative responses.

## RESULTS

### Effect of *xid* on polyclonal activation

Spleen cells from normal CBA/H (+/+) or immunodeficient CBA/N (*xid/xid*) mice were stimulated *in vitro* with PG, LPS, protein A or PWM, or left untreated. In separate experiments, groups of mice were left untreated (control) or were injected i.v. with a single dose of PG or LPS. Four days after the initiation of the cultures or *in vivo* injection, the numbers of IgM-, IgG- and IgA-secreting cells were assayed to measure total polyclonal activation. Cells secreting IgG1 and IgG2a, rather than all IgG subclasses, were assayed, because high levels of IgG1 and IgG2a are known to be present in the serum of some autoimmune mice and, therefore, may be important for the development of autoimmunity (Theofilopoulos *et al.*, 1983). Also, IgG1 and IgG2 responses are more T cell dependent than IgG3 and IgM responses (Park *et al.*, 1983) and, therefore, different mechanisms may be responsible for the changes in IgG1 and IgG2 than IgG3 and IgM responses. The numbers of cells secreting IgM antibodies to ssDNA and bromelin-treated autologous erythrocytes (HB MRBC) were used as a measure of polyclonal activation of autoantibody secreting cells, and cells secreting IgM antibodies to intact SRBC were measured as an indicator of polyclonal activation of specific antibodies which are not autoantibodies. Anti-SRBC antibodies are different from anti-MRBC antibodies and show less than 10% cross-reactivity with HB MRBC (Pages & Bussard, 1975).

In *in vivo* experiments, injections of PG or LPS significantly increased the numbers of cells per spleen in both normal ($81 \pm 7{\cdot}2$ *vs* $129 \pm 19$ or $109 \pm 6{\cdot}7$,

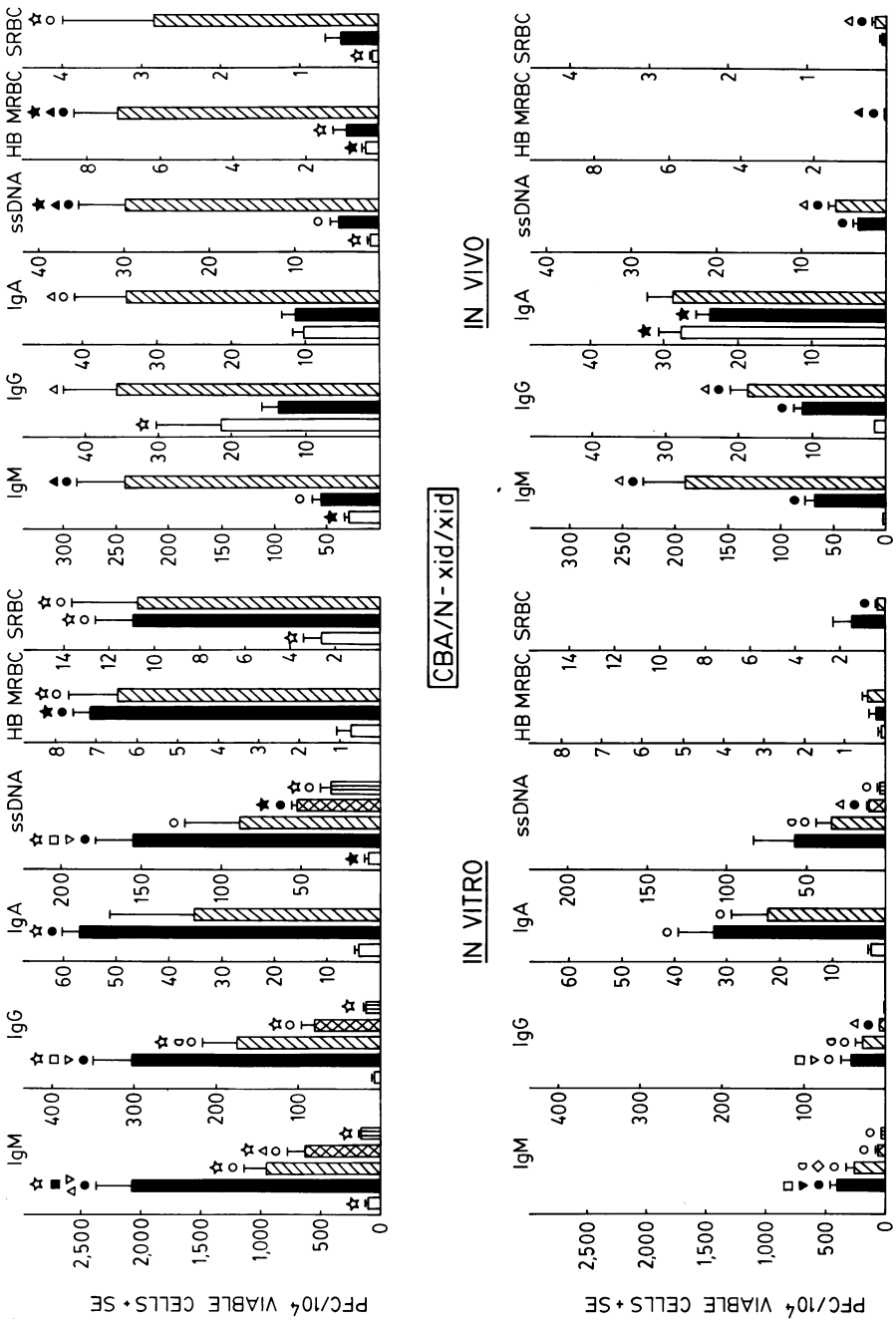


**Figure 1.** *In vitro* and *in vivo* polyclonal activation of IgM-, IgG (1+2a)-, IgA-, IgM anti-ssDNA, IgM anti-HB MRBC and IgM anti-SRBC-secreting PFC in CBA/H-+/+ and CBA/N-*xid*/*xid* mice. The values are means of three separate experiments in which $5 \times 10^5$ splenocytes were cultured for 4 days with PG (400 μg/ml), LPS (50 μg/ml), protein A (1000 μg/ml), PWM (1:100, v:v) or in medium alone (*in vitro*); or of seven mice per group injected i.v. 4 days before the assay with 40 mg/kg of PG or 10 mg/kg of LPS, or left untreated (*in vivo*). The open symbols indicate significantly higher values at $0{\cdot}005 < P < 0{\cdot}05$ and the closed symbols at $P < 0{\cdot}005$ between the following groups: ○●, PG, LPS, protein A or PWM *vs* nil; △▲, PG *vs* LPS, or protein A *vs* PWM; ▽▼, PG *vs* protein A; □■, PG *vs* PWM; ◇◆, LPS *vs* protein A; ○●, LPS *vs* PWM; ☆★, +/+ *vs* immunodeficient.

respectively, means ±SE) and *xid* (43±4·2 *vs* 112±6·8 or 85±11) mice. Untreated *xid* mice had significantly lower numbers of cells per spleen than normal mice. The differences between the numbers of cells per spleen in injected *xid* and normal mice were not significant.

The PFC responses (Fig. 1) were statistically analysed for the significance of differences in each type of the response (antibody class or specificity) between stimulated and unstimulated groups, between groups stimulated with each polyclonal activator and between *xid* and normal mice. Since our preliminary experiments indicated that protein A and PWM were much less effective polyclonal activators than PG and LPS, studies on protein A and PWM were limited to only the *in vitro* induction of polyclonal IgM-, IgG- and IgM anti-ssDNA antibody-secreting cells.

In most cases, the numbers of cells secreting all Ig and anti-DNA or anti-RBC antibodies could be increased by stimulation with polyclonal activators in both *xid* and normal mice (Fig. 1). *In vitro*, PG and LPS were equally strong polyclonal activators in both strains of mice, and PG and LPS were significantly stronger polyclonal activators than protein A and PWM in 83% (PG) and 42% (LPS) of the cases. Protein A was a significantly stronger polyclonal activator than PWM in 1/3 of the cases. *In vivo*, LPS was a significantly stronger polyclonal activator than PG in 83% of the cases in both *xid* and normal mice.

When the *in vitro* responses of *xid* and normal mice were compared, the numbers of cells secreting all Ig, anti-DNA and anti-RBC antibodies were higher in normal than in *xid* mice, and the differences were statistically significant in 80% of the cases (Fig. 1). The least affected responses were the IgA PFC. Autoantibody responses were not preferentially reduced (i.e. more than all Ig or heteroantibody responses) in *xid* mice, which suggests that low numbers of autoantibody-secreting cells in *xid* mice *in vitro* are due to a generally low response of these mice to all polyclonal B cell activators.

When the *in vivo* responses of *xid* and normal mice were compared (Fig. 1), the results were quite different than the *in vitro* results described above. *In vivo*, untreated normal mice had significantly higher numbers of all PFC than untreated *xid* mice, with the exception of IgA PFC which were significantly higher in *xid* mice. In fact, the numbers of IgA PFC in untreated *xid* mice were as high as in LPS-stimulated normal mice, and the numbers of IgA PFC in *xid* mice were not increased further following injection of polyclonal activators. When the numbers of PFC in LPS- or PG-injected *xid* and normal mice were compared, it became evident that the polyclonal IgM, IgG and IgA responses were similar in both strains (or even IgA PFC in PG-injected *xid* mice were significantly higher than in normal mice), whereas the anti-DNA and anti-RBC responses were, in most cases, significantly lower in injected *xid* than in injected normal mice (Fig. 1). This phenomenon was evident in all LPS-injected mice and was somewhat less pronounced in PG-injected mice, because PG, in contrast to LPS (as discussed above), is not a very effective polyclonal activator *in vivo*. These results show, therefore, preferential retardation of polyclonal anti-DNA and anti-RBC antibody responses by *xid* genes, i.e. these responses are severely reduced *in vivo* in polyclonally activated *xid* mice (when compared to normal mice), whereas the total polyclonal IgM, IgG and IgA responses are not.

### Effect of *xid* on frequencies of anti-DNA and anti-RBC antibody secreting cells

Based on the numbers of all IgM-secreting PFC (determined by the protein A PFC assay) and the numbers of cells secreting IgM specific to ssDNA, HB MRBC and SRBC, we determined the percentages of all IgM-secreting cells which produced antibodies specific to these antigens. Since we have observed some differences between total polyclonal Ig and anti-DNA and anti-RBC responses, our purpose was to determine if the frequencies of cells secreting these auto- or hetero-antibodies were different following *in vitro* or *in vivo* polyclonal activation in *xid* and normal mice. The summary of these results is shown in Table 1.

In both normal and *xid* mice, the *in vitro* frequencies of cells secreting anti-DNA, anti-HB MRBC and anti-SRBC antibodies were not significantly increased by polyclonal activation (Table 1), with one exception of protein A-induced anti-DNA response in *xid* mice. *In vivo*, the frequencies of cells secreting anti-DNA antibodies were significantly increased in both *xid* and normal mice following an injection of polyclonal activators, when compared to uninjected mice. When the *in vitro* and *in vivo* frequencies of cells secreting anti-DNA, anti-HB MRBC and anti-SRBC antibodies were compared, significantly higher *in vitro* and *in vivo* frequencies of these cells were seen in *xid* mice. In most cases, the *in vitro* and *in vivo* frequencies of these cells were not significantly different in normal (CBA/H) mice. However, this is not the case in all

**Table 1.** Frequencies (percentages) of cells secreting anti-ssDNA, HB MRBC and SRBC antibodies (out of all IgM-secreting cells) in polyclonal activation in CBA/H-+/+ and CBA/N-*xid*/*xid* mice*

| | | % of all PFC secreting IgM specific to: | | | | | |
|---|---|---|---|---|---|---|---|
| | | ssDNA | | HB MRBC | | SRBC | |
| Strain | Stimulant | *In vitro* | *In vivo* | *In vitro* | *In vivo* | *In vitro* | *In vivo* |
| CBA/H -+/+ | Nil | 7·9±3·0 | 3·8±0·83‡ | 0·87±0·19 | 1·4±0·41 | 2·9±1·0§ | 0·33±0·13 |
| | PG | 7·1±0·80 | 10·5±4·1 | 0·48±0·05 | 1·7±0·72 | 0·73±0·13 | 0·98±0·43 |
| | LPS | 8·3±2·4 | 15·7±4·8¶‡ | 1·0±0·21 | 3·5±0·98 | 1·8±0·51 | 1·7±0·87 |
| | Protein A | 11·1±4·5 | n.t. | n.t. | n.t. | n.t. | n.t. |
| | PWM | 18·3±2·9 | n.t. | n.t. | n.t. | n.t. | n.t. |
| | Mean† | 9·6±1·3 | 10·0±2·3‡ | 0·79±0·12** | 2·2±0·46** | 1·8±0·45‡ | 0·99±0·33‡ |
| CBA/N -*xid*/*xid* | Nil | 4·5±3·8 | 1·2±0·33 | <0·7 | 0·06±0·02 | <0·8 | 0·15±0·04 |
| | PG | 13·7±5·4†† | 4·8±0·37‡‡ | 0·05±0·03 | 0·015±0·008 | 0·35±0·17†† | 0·06±0·02 |
| | LPS | 14·7±6·2†† | 3·5±0·59¶ | 0·17±0·03§ | 0·038±0·009 | 0·17±0·05 | 0·09±0·015 |
| | Protein A | 16·3±1·9¶ | n.t. | n.t. | n.t. | n.t. | n.t. |
| | PWM | 10·1±1·3 | n.t. | n.t. | n.t. | n.t. | n.t. |
| | Mean† | 11·9±1·9§ | 3·2±0·41 | 0·11±0·03§ | 0·038±0·008 | 0·26±0·09§ | 0·10±0·02 |

* The values are means ±SE of the frequencies (percentages) from three *in vitro* experiments. The absolute numbers of PFC and experimental details are given in Fig. 1; n.t., not tested.

† Mean of unstimulated and all stimulated groups.

‡§¶**†††‡‡ Significantly higher values at $0{\cdot}005 < P < 0{\cdot}05$ (¶†††) or at $P < 0{\cdot}005$ (‡‡§**) between the following groups: ‡** +/+ *vs* immunodeficient; ¶‡‡ stimulated *vs* unstimulated; ††§ *in vitro vs in vivo*.

normal mice, in which the frequencies of these cells *in vitro* are often higher than *in vivo* (see the results on BALB/c mice below and on BALB/c and C57BL/6 mice in R. Dziarski, submitted for publication).

When the frequencies of cells secreting anti-DNA, anti-HB MRBC and anti-SRBC antibodies in *xid* and normal mice were compared (Table 1), higher frequencies of these cells were observed in normal than in *xid* mice (with statistically significant differences for the pooled results for all groups in each strain). The only exceptions were the *in vitro* frequencies of anti-DNA antibody secreting cells, which were similar in *xid* and normal mice.

In summary, these results indicate that in *xid* mice the frequencies of cells secreting anti-DNA antibodies were lower than normal only in *in vivo* (but not in *in vitro*) polyclonal activation, and the frequencies of cells secreting anti-RBC antibodies were lower in *xid* mice both *in vivo* and *in vitro*.

### Effect of *nu* on polyclonal activation

*In vitro* and *in vivo* experiments, analogous to those described in the first section of the Results, were performed on immunodeficient athymic BALB/c (*nu*/*nu*) mice and normal euthymic BALB/c (+/+) mice as controls. *In vivo*, injections of PG significantly increased the numbers of cells per spleen in both normal (128±8·6 *vs* 188±21, means ±SE) and nude (56±5·6 *vs* 116±22) mice, whereas injections of LPS resulted in a significant increase in the numbers of cells per spleen only in nude (103±11), but not in normal (134±17) BALB/c mice. Nude mice had lower numbers of cells per spleen than normal mice, and the differences were statistically significant in untreated and PG-injected mice.

In most cases, *in vitro* or *in vivo* polyclonal activation significantly increased the numbers of Ig-, anti-DNA and anti-RBC antibody-secreting cells in both normal and nude mice (Fig. 2). In normal BALB/c mice *in vitro*, PG was in most cases a stronger polyclonal activator than LPS (in four out of six assays significantly stronger), whereas protein A and PWM induced lower polyclonal activation responses than PG or LPS. *In vivo*, in normal BALB/c, LPS was a stronger stimulant than PG (significantly stronger in half of the assays). Similar results were obtained in nude mice, but the differences between PG and LPS

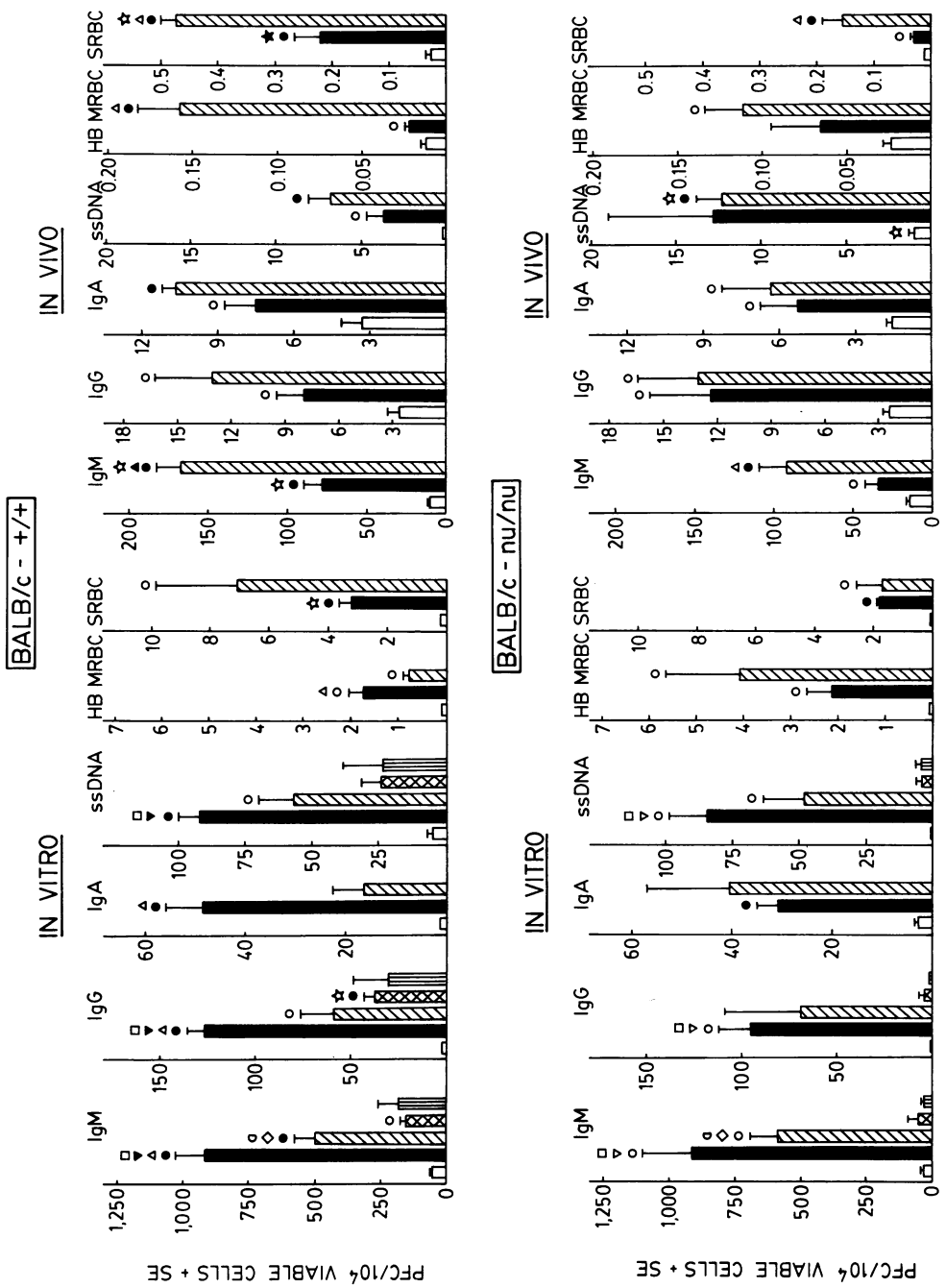


**Figure 2.** *In vitro* and *in vivo* polyclonal activation in BALB/c-+/+ and BALB/*c-nu/nu* mice. All details are the same as in Fig. 1, except that in *in vivo* experiments, five to seven mice per group were used.

**Table 2.** Frequencies (percentages) of cells secreting anti-ssDNA, HB MRBC and SRBC antibodies (out of all IgM-secreting cells) in polyclonal activation in BALB/c-+/+ and BALB/c-*nu/nu* mice*

| | | % of all PFC secreting IgM specific to: | | | | | |
|---|---|---|---|---|---|---|---|
| | | ssDNA | | HB MRBC | | SRBC | |
| Strain | Stimulant | *In vitro* | *In vivo* | *In vitro* | *In vivo* | *In vitro* | *In vivo* |
| BALB/c -+/+ | Nil | 8·6±2·0§ | 2·0±0·31 | 0·16±0·04 | 0·11±0·03 | 0·40±0·07 | 0·26±0·08 |
| | PG | 10·3±1·2†† | 4·4±0·96¶ | 0·19±0·01§ | 0·03±0·005 | 0·36±0·04 | 0·33±0·12 |
| | LPS | 11·0±2·1†† | 3·9±0·47¶ | 0·17±0·05 | 0·09±0·008 | 1·29±0·5†† | 0·29±0·05 |
| | Protein A | 12·7±5·4 | n.t. | n.t. | n.t. | n.t. | n.t. |
| | PWM | 9·9±3·9 | n.t. | n.t. | n.t. | n.t. | n.t. |
| | Mean† | 10·6±1·2§ | 3·4±0·42 | 0·17±0·02§ | 0·09±0·002 | 0·68±0·21†† | 0·28±0·05** |
| BALB/c *-nu/nu* | Nil | 4·2±0·25 | 7·5±2·4‡ | 0·41±0·12 | 0·18±0·04 | <0·61 | 0·09±0·01 |
| | PG | 8·1±2·2 | 30·8±10¶‡ | 0·26±0·08 | 0·19±0·09 | 0·21±0·05 | 0·10±0·03 |
| | LPS | 7·8±2·7 | 18·0±3·9¶** | 0·58±0·32 | 0·18±0·07 | 0·34±0·21 | 0·19±0·02‡‡ |
| | Protein A | 10·3±3·2 | n.t. | n.t. | n.t. | n.t. | n.t. |
| | PWM | 13·7±7·4 | n.t. | n.t. | n.t. | n.t. | n.t. |
| | Mean† | 8·8±1·7 | 18·7±4·3**†† | 0·42±0·11‡†† | 0·18±0·03‡ | 0·29±0·07†† | 0·13±0·02 |

* The values are means ±SE of the frequencies (percentages) from three *in vitro* experiments, or five to seven mice per each (uninjected or injected) group in *in vivo* experiments. The absolute numbers of PFC and experimental details are given in Fig. 2; n.t., not tested.

For explanation of other footnotes, see Table 1.

were less pronounced and the protein A- and PWM-induced responses were lower than in normal mice.

Very few statistically significant differences were observed when the *in vitro* polyclonal antibody and autoantibody responses of nude and normal BALB/c mice were compared (Fig. 2). When the *in vivo* responses of athymic and euthymic mice were compared, significantly higher numbers of total IgM PFC and anti-SRBC PFC were seen in normal than in nude mice, and significantly higher anti-DNA PFC responses were seen in nude than in normal mice (Fig. 2).

These results, therefore, indicate preferential enhancement of anti-DNA polyclonal antibody responses in athymic nude mice *in vivo* (but not *in vitro*), as compared to the responses in normal euthymic mice.

### Effect of *nu* on frequencies of anti-DNA and anti-RBC antibody secreting cells

In both nude and normal mice, the *in vitro* frequencies of cells secreting anti-DNA, anti-HB MRBC and anti-SRBC antibodies were not significantly increased by polyclonal activation (Table 2). *In vivo*, the frequencies of cells secreting anti-DNA antibodies were significantly increased in both normal and nude mice following an injection of PG or LPS, when compared to the untreated mice. The frequency of anti-SRBC antibody-secreting cells was also increased in nude mice following an injection of LPS.

In normal BALB/c mice, the *in vitro* frequencies of all cells secreting all antibodies assayed were significantly higher than the corresponding *in vivo* frequencies (Table 2). By contrast, in nude mice, significantly higher *in vivo* than *in vitro* frequencies of anti-DNA antibody-secreting cells were observed, whereas the frequencies of cells secreting anti-HB MRBC and anti-SRBC antibodies were (similarly to normal mice) higher *in vitro* than *in vivo*.

When the frequencies of cells secreting anti-DNA, anti-HB MRBC and anti-SRBC antibodies in nude and normal mice were compared (Table 2), significantly higher *in vivo* frequencies of anti-DNA and anti-HB MRBC antibody-secreting cells were found in nude than in normal mice. The frequencies of anti-SRBC antibody-secreting cells were higher in normal than in nude mice.

In conclusion, these results show similar *in vitro* frequencies of anti-DNA and anti-RBC antibody-sec-

**Table 3.** *In vitro* mitogenic responses of splenocytes from CBA/H-+/+, CBA/N-*xid*/*xid*, BALB/c-+/+ and BALB/*nu*/*nu* mice*

| Mitogen | Mean c.p.m. ± SE† | | | |
|---|---|---|---|---|
| | CBA/H-+/+ | CBA/N-*xid*/*xid* | BALB/c-+/+ | BALB/c-*nu*/*nu* |
| Nil | 954 ± 46‡ | 231 ± 31 | 903 ± 67 | 1199 ± 188 |
| PG | 59,916 ± 1156‡ | 13,120 ± 1133 | 26,512 ± 2038 | 47,131 ± 5088‡ |
| LPS | 61,016 ± 2056‡ | 19,583 ± 833 | 26,008 ± 1802 | 39,787 ± 5459§ |
| Protein A | 63,734 ± 2181‡ | 27,040 ± 1962 | 56,310 ± 5061‡ | 7434 ± 760 |
| PWM | 9676 ± 624 | 7920 ± 843 | 10,486 ± 870‡ | 2164 ± 155 |

* $2 \times 10^5$ cells were cultured with the indicated mitogens (PG, 400 μg/ml; LPS, 100 μg/ml; protein A, 1000 μg/ml; PWM, 1:100, v:v) for 24 hr and pulsed with [$^3$H] thymidine for an additional 18 hr.

† The values are means determined from 12 cultures in 3 experiments.

‡ Significantly higher values (+/+ *vs xid*/*xid* or +/+ *vs nu*/*nu*) at $P < 0{\cdot}001$.

§ Significantly higher values (+/+ *vs xid*/*xid* or +/+ *vs nu*/*nu*) at $0{\cdot}01 < P < 0{\cdot}02$.

reting cells in nude and normal mice, and significantly higher *in vivo* frequencies of anti-DNA and anti-HB MRBC antibody-secreting cells in nude than in normal mice.

### Effects of *xid* and *nu* on mitogenic responses

Because different polyclonal activators can induce strong mitogenic responses and weak polyclonal antibody responses or vice versa in different hosts (reviewed in Dziarski, 1982b, 1982c; McKearn *et al.*, 1982), we decided to test if the *xid* or *nu* mutations also had depressive or enhancing effects on mitogenic responses induced by the same polyclonal activators that were used in the studies on induction of polyclonal antibodies. In *xid* mice, the *in vitro* mitogenic responses to PG, LPS and protein A were lower than normal (Table 3), whereas, as indicated previously, all four stimulants induced low *in vitro* polyclonal antibody responses. In nude mice, mitogenic responses to PG and LPS were higher, and to protein A and PWM, lower than normal (Table 3). As previously indicated, *in vitro* polyclonal antibody responses to PG and LPS were similar in nude and normal mice and, to protein A and PWM, lower in nude than in normal mice.

## DISCUSSION

Our results confirm and extend previous observations (discussed in the Introduction) that the two immunodeficiencies, expressed in homozygous *xid* or *nu* mice, have opposing effects on polyclonal B cell activation: *xid* results in retarded and *nu* results in enhanced or unchanged polyclonal antibody and autoantibody production and cell proliferation. This general conclusion, however, as shown by our results, is an oversimplification, because the final outcome of polyclonal activation of B cells from these immunodeficient mice also depends on the *in vitro* or *in vivo* conditions of the stimulation, the type of polyclonal activator used and the type of antibody assayed (isotype and specificity). Even though we have studied the *xid* and *nu* mutations in two different strains of mice, valid comparisons can be made, since the expression of these genes is similar in most normal and autoimmune strains (Milich & Gershwin, 1980; Pisetsky, Caster & Steinberg, 1983; Scher, 1982; Smith *et al.*, 1983a).

The most interesting are the differences in *in vitro* and *in vivo* induced responses. Our results confirm previous reports of low *in vitro* responses of *xid* mice to LPS, PWM and protein A (Huber & Melchers, 1979; McKearn *et al.*, 1982; Perlmutter *et al.*, 1970; Scher, 1982; Tanabe, Mochizuki & Nakano, 1981) and show that PG-induced responses are similarly affected. Since stimulation with all polyclonal activators results in similarly reduced numbers of cells secreting autoantibodies, hetero-antibodies and all polyclonal Ig, it can be concluded that autoantibody responses are not preferentially reduced by the *xid* mutation *in vitro*. These results are consistent with the recent results of Pisetsky *et al.* (1983) who showed parallel reduction in the frequencies of anti-DNA and anti-TNP precursors

in CBA/N (*xid*/*xid*) and DBA/2 (*xid*/*xid*) mice *in vitro*. From these results, it can be concluded that low numbers of autoantibody-secreting cells in *xid* mice *in vitro* are due to generally depressed *in vitro* responsiveness to polyclonal B cell activators. It has been suggested before that this low responsiveness is due to the lack or low frequency of mitogen-responsive B cells, which belong to the Lyb $3^+$, $5^+$, $7^+$ subpopulation (Huber & Melchers, 1979; Pisetsky *et al.*, 1983; Scher, 1982).

Quite different results, however, have been obtained *in vivo*. Our data show that the deficit in polyclonal antibody responses that is seen *in vitro* in *xid* mice can be partially overcome *in vivo*. Although in unstimulated *xid* mice *in vivo* the numbers of cells secreting total IgM, IgG (1 + 2a), and anti-DNA and anti-RBC antibodies were lower than those in unstimulated normal mice, a single injection of a polyclonal activator could increase the numbers of IgM- and IgG-secreting cells to the level seen in stimulated normal mice. This effect, however, was not seen with cells secreting IgM anti-DNA and IgM anti-RBC antibodies, which were still selectively suppressed in polyclonally activated *xid* mice, as compared to the numbers of these cells seen in *in vivo* polyclonal activation of normal mice or polyclonal activation of all Ig-secreting cells in *xid* mice. These results indicate that different mechanisms regulate polyclonal activation of total IgM- and IgG-secreting cells than of specific anti-DNA and anti-RBC antibody-secreting cells *in vitro* and *in vivo*. Furthermore, these results indicate that the low polyclonal anti-DNA and anti-RBC responses in *xid* mice *in vivo* are not due to general deficiency of these mice to produce polyclonal Ig *in vivo*.

In all normal strains of mice studied by us so far (Dziarski, 1982a, 1982b; R. Dziarski, submitted for publication, and this paper), the numbers of antibody-secreting cells (per million of viable cells) are several times higher *in vitro* than *in vivo*. Normal mice, therefore, preferentially develop antibody- and autoantibody-secreting cells in polyclonal activation *in vitro*, as compared to polyclonal activation *in vivo*. As shown in this paper, this difference between the *in vitro* and *in vivo* polyclonal antibody responses is much smaller in *xid* mice for total Ig-secreting cells (but not for autoantibody-secreting cells), due to a relative inability of *xid* mice to mount high polyclonal antibody responses *in vitro*, and because of their good polyclonal antibody responsiveness *in vivo*. The reasons for these differences are not clear.

Also unexplained are the reasons for the higher than normal numbers of IgA-secreting cells in unstimulated *xid* mice, which were as high as the numbers of IgA-secreting cells in normal mice following polyclonal activation. This phenomenon may be related to the presence of mature B cells (which are able to mount normal immune responses) in the Peyer's patches of young *xid* mice (Eldridge *et al.*, 1983). It is, therefore, possible, that the IgA-secreting cells in the spleens of *xid* mice originate from Peyer's patches or other lymphoid organs active in local immune responses.

Athymic nude and euthymic BALB/c mice had similar *in vitro* polyclonal antibody and autoantibody responses. By contrast, *in vivo*, nude mice had higher numbers of IgM anti-DNA antibody-secreting cells than normal mice, even though the total numbers of IgM-secreting cells and anti-SRBC antibody-secreting cells were lower in nude than in normal mice. This indicates preferential induction of anti-DNA antibody-secreting cells in nude mice *in vivo*, as compared to the numbers of these cells in normal mice, or the induction of all polyclonal IgM in nude mice. The numbers of IgG (1 + 2a)-, IgA- and anti-HB MRBC antibody-secreting cells were similar in nude and normal mice *in vivo*, which contradicts previous findings of McKearn *et al.* (1982), who showed depressed IgG (1 + 2) responses in polyclonal activation in nude mice. The reason for this difference is not clear.

Our results on preferential induction of anti-DNA antibodies *in vivo* in nude mice support the conclusions of Smith *et al.* (1983b), who demonstrated that the combination of polyclonal activation and thymectomy could most easily induce autoimmunity. Our results also explain previous seemingly contradictory observations showing the lack of enhancement of autoimmune responses *in vitro* in T cell-depleted cultures (Primi *et al.*, 1978), which was taken as evidence for the lack of a role for suppressor T cells in prevention of autoimmunity and maintenance of self-tolerance. Smith *et al.* (1983b), however, concluded that, in their system, the induction of autoimmunity in genetically non-autoimmune strains was facilitated by a failure of normal suppressor function and abnormal presence of contrasuppression. Since these defects in suppressor and contrasuppressor function could be demonstrated both *in vivo* and *in vitro* (Smith *et al.*, 1983b), they are an unlikely explanation for the differences in the relative ease of induction of anti-DNA antibodies in nude mice in our experiments, that was only seen *in vivo* but not *in vitro*.

Further experiments are needed to elucidate the mechanism responsible for these differences.

The enhancement of anti-DNA autoantibody responses *in vivo* in nude BALB/c mice reported in this paper, is consistent with previous data that showed a worsening of autoimmunity in athymic (*nu/nu*) New Zealand (Milich & Gershwin, 1980) and BXSB (Smith *et al.*, 1983a) mice. This indicates that similar basic mechanisms are responsible for the regulation of autoimmune responses in normal and genetically autoimmune-prone mice.

The opposing effect of *xid* and *nu* mutations on polyclonal activation of autoantibodies is also well illustrated by the differences between the frequencies of autoantibody-secreting cells in *xid* or nude and normal mice. While the *in vitro* frequencies of anti-DNA antibody-secreting cells were similar in all strains of mice, the *in vivo* frequencies of these cells were significantly reduced in *xid* mice and significantly increased in nude mice, as compared to normal mice. In nude mice, this effect (to a lesser extent) was also seen with anti-HB MRBC antibody-secreting cells, whereas the frequencies of cells secreting anti-SRBC antibodies were lower than normal. In *xid* mice, however, lower than normal frequencies of both anti-HB MRBC and anti-SRBC antibody-secreting cells were seen both *in vivo* and *in vitro*.

The results presented in this paper extend our previous observations (Dziarski, 1980; Dziarski, 1982a, 1982b; R. Dziarski, submitted for publication) showing that PG is an equally strong or stronger polyclonal activator than LPS *in vitro*, whereas *in vivo*, LPS is more effective than PG. Also, PG and LPS were more effective polyclonal activators *in vitro* than protein A and PWM. This pattern of responsiveness was observed by us before in three normal and four genetically autoimmune strains of mice (Dziarski, 1980; Dziarski, 1982a, 1982b; R. Dziarski, submitted for publication), and now it has been extended to *xid* and nude mice. Since PG-induced *in vitro* responses in *xid* mice were similarly diminished as the LPS-induced responses, these data indicate that PG, as with LPS, activates primarily late-maturing, Lyb $3^+$, $5^+$, $7^+$ B cells.

The reasons for the difference between the *in vivo* and *in vitro* polyclonal activating ability of PG and LPS, which has been observed in all strains of mice so far tested and was most prominent in genetically autoimmune-prone strains of mice (R. Dziarski, submitted for publication), are presently not known. Possible differences between the B cell subpopulations activated by these two polyclonal activators are an unlikely explanation for these differences, because PG and LPS are equally active in the polyclonal activation of the cells from the same animals *in vitro*. One possible explanation is that PG-induced polyclonal activation *in vivo* is more sensitive than LPS-induced polyclonal activation to some regulatory influences which are less active in normal than in autoimmune mice *in vivo* and still less active or inactive *in vitro*. Another possible explanation could be that PG is removed faster than LPS *in vivo* by the phagocytic cells, thus having less opportunity to activate lymphocytes. This explanation is less likely, however, because PG was as active or more active than LPS in the induction of *in vivo* proliferation of splenocytes in normal, immunodeficient and autoimmune mice (Results, and R. Dziarski, submitted for publication). Also, phagocytic cells show enhanced activity in old but not in young autoimmune mice (Watanabe & Russell, 1980), whereas both young and old autoimmune mice were unresponsive to *in vivo* polyclonal activation by PG (R. Dziarski, submitted for publication). Elucidation of these differences will certainly shed more light on the *in vitro* and *in vivo* mechanisms of B cell activation and induction of autoantibodies.

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