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Differential tolerance of thymus-independent and thymus-dependent antibody responses

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Summary. The effect of pre-immunization with soluble haptenated serum proteins or haptenated carbohydrates on the subsequent responses to the hapten in thymus-dependent or thymus-independent form was investigated. In certain cases both thymus-dependent and -independent responses were suppressed whilst in others a differential suppression of thymus-independent responsiveness was observed. These results are discussed in terms of B-cell subpopulations and mechanisms of tolerance induction.

INTRODUCTION

In recent years evidence has been accumulating regarding B-cell heterogeneity with respect to susceptibility to T-cell regulation. Playfair & Purves (1971) suggested, on the basis of anatomical distribution, that two subpopulations of B cell exist in normal animals, one of which can only generate antibody-forming cells with the help of T lymphocytes (B2 cells), and another which is functionally thymus-independent in this respect (B1 cells). Other groups have produced data consistent with this hypothesis. The two subpopulations are thought to differ in terms of size (Gorczinski & Feldmann, 1975), drug sensitivity (Galanaud, Crevon & Dormont, 1976) and expression of the C3 receptor (Arnaiz-Villena; Lewis, Ranken, Nitecki & Goodman, 1976). More recently Jennings & Rittenberg (1976) have shown additive stimulatory effects when cultures are challenged simultaneously with thymus-dependent and thymus-independent antigens. Also, a substrain of CBA/H mice, designated CBA/N, has been shown to be deficient with regard to responsiveness to many thymus-independent antigens, whilst responses to thymus-dependent antigens are relatively normal (Cohen, Scher & Mosier, 1976).

In this communication, we report on studies generated by previous experiments in this laboratory on tolerance induction, which appear to be consistent with the B1/B2 hypothesis. By two different tolerization regimes we show differential suppression of thymus-independent responsiveness whilst responsiveness to thymus-dependent antigens remains unaffected.

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MATERIALS AND METHODS

Animals

(BALB/c × C57 B1)F_1, (BALB/c × CBA/H)F_1 and BALB/c mice were bred in our own laboratories from inbred stocks obtained from the Laboratory Animals Centre, Carshalton. Male and female mice were

used between the ages of 2 and 5 months. Non-inbred nude (nu/nu) mice and their heterozygous (nu/+) littermates were obtained from Olac Southern, Blackthorn, Oxford.

Irradiation

Female (BALB/c × CBA/H)F_1 mice were given 750 rad of X-rays at a dose rate of approximately 30 rad/min using a Marconi 250 kV X-ray machine.

Antigens and immunization

Protein antigens were dinitrophenylated according to the method described by Little & Eisen (1967). Briefly, 100 mg of protein and 100 mg of dinitrobenzenesulphonic acid (DNBS) were dissolved in phosphate buffered saline (PBS) containing 2% potassium carbonate, and stirred gently for 18 h at room temperature. Unreacted DNBS was removed by separation on a Sephadex G-25 column followed by extensive dialysis against PBS. Chicken and mouse gamma globulins were prepared from normal sera by 40% ammonium sulphate precipitation. Keyhole limpet haemocyanin (KLH) (mol. wt 4×10^6) was obtained from Calbiochem, San Diego California. Bovine serum albumin (mol. wt. 59,000) was obtained from Armour Pharmaceuticals Ltd. Eastbourne, England.

Carbohydrates were dinitrophenylated according to the method of Axen, Porath & Ernbach (1967). Ficoll (mol. wt 400,000) and dextran (mol. wt 250,000) were obtained from Pharmacia Fine Chemicals A.B., Uppsala, Sweden. Dinitrophenylated pneumococcal polysaccharide type III (DNP-SIII) was a kind gift from Dr G. G. B. Klaus. The following conjugates were prepared, giving the number of DNP groups per carrier molecule as determined by spectrophotometric and dry weight analysis: DNP_{29} CGG, DNP_{23} MGG, DNP_{350} KLH, DNP_6BSA, DNP_{38} Ficoll, DNP_{15} Ficoll, DNP_{14} Ficoll and DNP_{40} dextran.

For immunization, DNP-CGG and DNP-KLH were alum precipitated and given intraperitoneally, usually with 2×10^9 *Bordetella pertussis* organisms (Lister Institute, Elstree). The assay for plaque forming cells (see below) was performed 7 days after immunization. DNP-Ficoll and DNP-dextran were administered intraperitoneally in saline, the PFC assay being performed 4 or 5 days after immunization. For tolerance induction conjugates were administered in saline. Groups of 4–6 animals were immunized and the results of the PFC assay expressed as geometric means ± s.e.

Cell cultures

Dissociated spleen cells were cultured in the presence or absence of added antigen in 50 mm petri dishes (Sterilin Products, Teddington, England). Individual cultures contained 3×10^7 cells at a concentration of 10^7 cells/ml. RPMI 1640 (Flow Laboratories, Northampton) containing 5 mM HEPES, 10% Fetal calf serum (Flow) and added glutamine was used as tissue culture medium. Cultures were incubated for 72 h at 37° in a humidified atmosphere containing 10% CO_2.

PFC assay

Assays for plaque-forming cells were performed as previously described (Marshall-Clarke & Playfair, 1975).

Table 1. Tolerance induction by haptenated isologous gammaglobulin

Pretreatment*	Thymus independent response†		Thymus dependent response‡	
	Direct PFC/spleen	Indirect PFC/spleen	Direct PFC/spleen	Indirect PFC/spleen
Nil	17,059 (14,120–20,609)	9814 (7238–13,308)	35,554 (27,194–46,484)	158,281 (140,682–178,082)
1 mg DNP MGG	1093 (722–1652)	Nil	2921 (2088–4080)	7560 (6477–8822)

* (BALB/c × C57B1)F1 mice were pretreated as indicated 7 days before challenge.
† Mice challenged with 25 μg DNP_{38} Ficoll i/p.
‡ Mice challenged with 300 μg DNP-CGG (alum + *B. pertussis*) i/p).

Table 2. Tolerance induction by haptenated heterologous serum proteins

Expt. no.	Pretreatment	Thymus-independent response[2]		Thymus-dependent response[3]	
		Direct PFC/spleen	Indirect PFC/spleen	Direct PFC/spleen	Indirect PFC/spleen
1	Nil	38,224 (28,802–50,720)	15,294 (11,506–20,329)	22,738 (17,666–29,266)	47,559 (33,041–68,455)
	200μg DNP-CGG	11,339* (8929–14,400)	6668† (5476–8120)	26,688 (22,046–32,038)	45,808 (37,675–55,696)
2	Nil	47,713 (41,273–53,915)	32,285 (26,783–38,798)	34,318 (24,116–48,833)	117,663 (103,743–133,321)
	200 μg DNP-BSA	12,231‡ (11,076–24,176)	9095§ (4253–19,447)	47,132 (40,090–55,410)	137,168 (117,148–160,609)

1. (BALB/c × C57B1)F1 mice were pretreated as indicated 7 days before challenge.
2. Mice were challenged with 10μg DNP_{38} Ficoll in both experiments.
3. In exp. 1 mice were challenged with 250μg DNP-KLH alum + *B. pertussis* in exp. 2 mice were challenged with 200μg DNP-CGG alum + *B. pertussis*.

*0·005> P> 0·0025; †0·025> P> 0·0125; ‡0·01> P> 0·005; §0·05> P> 0·025 (by Student's t-test)

RESULTS

Induction of unresponsiveness by DNP-conjugates of isologous and heterologous serum proteins

In preliminary studies (BALB/c × C57Bl)F_1 mice were tolerized with haptenated isologous gamma globulins (DNP-MGG) and subsequently challenged with thymus-dependent or thymus-independent immunogens. Both responses were significantly suppressed by prior administration of tolerogen (Table 1). This result is consistent with the receptor blockade hypothesis suggested for the induction of unresponsiveness by non-immunogenic molecules (Aldo-Benson & Borel, 1974). It is suggested that passive blockade of membrane receptors prevents B-cell activation, thus it seems unlikely that any discrimination between thymus-dependent and thymus-independent antigens should occur (Table 1). However, if one considers the putative B1 cell, which cannot respond to antigen in a thymus-dependent form, an interesting paradox emerges. The binding of a thymus-dependent antigen by a B1 cell is operationally the same as the binding of non-immunogenic molecules by B1 or B2 cells as far as T cell intervention is concerned, as by definition the B1 cell cannot co-operate with T cells. Thus one might expect interaction of B1 cells with thymus-dependent antigens to induce tolerance. This provided the basis for the following experiments.

(BALB/c × C57B1)F_1 mice were pre-injected with either soluble DNP-CGG or soluble DNP-BSA as indicated in Table 2. They were challenged 7 days later with either DNP_{38}-Ficoll, a thymus-independent antigen (Mosier, Johnson, Paul & McMaster, 1974), or a non-homologous thymus-dependent antigen; DNP-KLH or DNP-CGG. As shown in Table 2, the prior administration of soluble DNP-protein conjugates differentially reduced the res-

Table 3. *In vitro* tolerance induction by DNP-CGG

Pretreatment of Cells	No. cells*	Direct PFC/spleen	Indirect PFC/spleen
Cultured alone for 72 h	3×10^7	14,248 (11,985–16,937)	13,122 (10,345–16,645)
Cultured with 200 μg/ml DNP-CGG for 72 h	3×10^7	2929† (2086–4412)	4625‡ (3883–5508)

*Cells transferred into lethally irradiated (BALB/c × CBA)F1 mice and challenged with 50 μg DNP_{38} Ficoll. PFC assay on day 6.

†0·0025> P> 0·0050; ‡0·0025> P> 0·0050.

ponse to the thymus-independent antigen without affecting the response to the thymus-dependent antigen (Table 2).

We considered that this result may indicate a functional difference between putative B-cell subpopulations. However, another explanation could be that a small amount of anti-hapten antibody is produced in response to the soluble DNP-protein conjugate and that this is sufficient to absorb the small soluble thymus-independent challenge but not the larger dose of particulate antigen that constituted the thymus-dependent challenge. This seemed unlikely because very little antibody is produced in response to soluble serum proteins. However, to ensure that antibody played no role we decided to attempt to induce tolerance *in vitro* and transfer the tolerized cells to irradiated recipients. Spleen cells were cultured for 72 h in the presence or absence of 200 μg/ml DNP-CGG. After thorough washing to remove any antibody accumulated during the culture period, the cells were transferred to lethally irradiated recipients which were challenged with DNP_{38} Ficoll. Table 3 shows that mice receiving cells pre-exposed to DNP-CGG gave reduced responses to DNP_{38} Ficoll compared to animals receiving cells cultured alone. Thus, it seems likely that interaction with cell surface receptors is responsible for the observed unresponsiveness (Table 3).

If this proposed cell surface interaction were to result in receptor blockade, the onset of tolerance should be immediate. Administration of DNP-MGG simultaneously with or after immunization results in significant suppression of responsiveness, both to thymus-dependent and thymus-independent antigens (Table 4 and Tite, unpublished observations). Preliminary experiments indicated that the simultaneous administration of soluble DNP-CGG and DNP_{38} Ficoll did not result in a suppression of the anti-DNP PFC response (Table 4). A more detailed study of the onset of unresponsiveness was therefore performed. (BALB/c × C57B1)F_1 mice were given soluble DNP-CGG at varying intervals before challenge with DNP_{38} Ficoll (Fig. 1). Only when the soluble thymus-

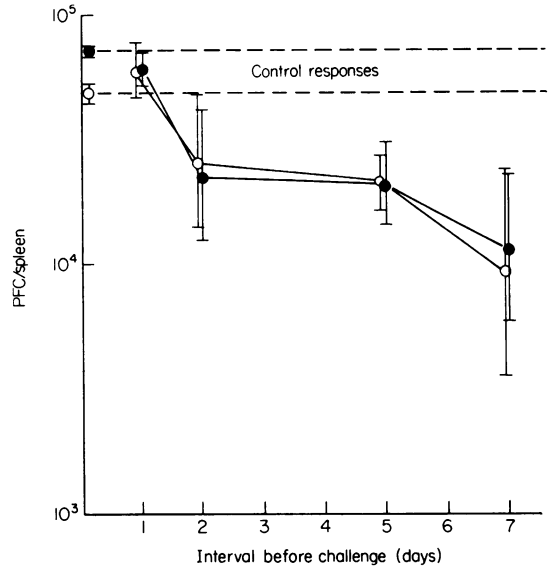

Figure 1. Time course of the onset of suppression induced by DNP-CGG. Responses of (BALB/c × C57B1)F1 mice to 10 μg DNP_{38}Ficoll at varying times after the administration of 100 μg soluble DNP-CGG. (●) Direct PFC; (○) Indirect PFC.

Table 4. Effect of simultaneous injection of haptenated serum proteins on the response to DNP-Ficoll

Pretreatment	Direct PFC/spleen	Indirect PFC/spleen
Nil	34,999 (25,822–47,437)	17,326 (13,582–21,871)
100 μg DNP MGG	9373 (6985–12,577)	727 (469–1126)
100 μg DNP-CGG	38,669 (33,445–44,708)	23,882 (23,365–24,411)

(BALB/c × CBA)F1 mice were injected intraperitoneally with soluble antigen as indicated above, or left as controls. All groups were simultaneously challenged with 10μg DNP_{38} Ficoll intraperitoneally. The PFC assay was performed five days after immunization.

Table 5. Induction of tolerance by DNP-CGG in nude mice

Genotype	Pretreatment	Direct PFC/spleen	Indirect PFC/spleen
nu/nu	Nil	15,563 (6763–35,810)	8419 (3,536–20,043)
nu/nu	250 μg DNP-CGG	2650 (1462–4801)	1914 (1,156–3,169)
nu/+	Nil	14,250 (11,687–17,376)	9247 (8,210–10,413)
nu/+	250μg DNP-CGG	5376 (4,002–7220)	5548 (3984–7725)

* Mice were challenged with 50 μg DNP_{15} Ficoll seven days after pretreatment.

dependent conjugate was given 48 h or more before the thymus-independent antigen was significant suppression observed. Similar results have been obtained using DNP-BSA as the tolerogen (unpublished observations). These kinetics are not consistent with a passive receptor blockade, indeed an active mechanism seems a more likely explanation. It seemed possible that carrier reactive T cells could be modulating the response of the B1 cell in a negative fashion, i.e. a T-cell mediated suppression. Therefore, the possible role of T cells in the induction of unresponsiveness was examined. Nude mice and their heterozygous littermates were preimmunized with 250 μg DNP-CGG. Seven days later both groups plus uninjected controls were challenged with 50 μg DNP_{15} Ficoll. The absence of T cells did not affect the reduction of the T-independent response (Table 5). Thus, if the induction of unresponsiveness is an active phenomenon, as suggested by the delayed onset, it seems unlikely to be a T-cell mediated process (Table 5).

Induction of unresponsiveness by DNP-conjugates of carbohydrate antigens

The induction of tolerance by large polymeric thymus-independent antigens has been well documented (Feldmann, 1972); Klaus & Humphrey, 1975). We decided to investigate two polymeric carbohydrate antigens in terms of tolerogenicity for thymus-dependent and thymus-independent responses. In our hands the DNP-conjugate of pneumococcal polysaccharide, DNP-SIII, is a poorly immunogenic molecule producing about 1000 direct PFC-spleen. DNP_{38} Ficoll, however, is highly immunogenic in (BALB/c × C57B1)F1 mice giving

Table 6. Induction of tolerance by haptenated carbohydrates

Expt no.	Pretreatment*	Thymus-independent response†		Thymus-dependent response‡	
		Direct PFC/spleen	Indirect PFC/spleen	Direct PFC/spleen	Indirect PFC/spleen
1	Nil	55,408 (48,107–63,816)	38,727 (31,281–47,943)	59,252 (52,855–66,421)	151,546 (109,954–208,809)
	250 μg DNP-SIII	303 (255–359)	230 (200–263)	958 (398–2304)	1314 (563–3063)
2	Nil	40,345 (39,156–41,576)	19,400 (16,326–23,052)	7,006 (4810–10,206)	51,549 (43,716–60,787)
	1 mg DNP_{38} Ficoll	5687 (4186–7726)	357 (258–492)	27,393 (24,649–30,442)	46,814 (41,288–53,079)

* In exp. 1 mice were pretreated with DNP-SIII seven days before challenge, in exp. 2 mice were pretreated with DNP_{38}Ficoll 21 days before challenge.

†Mice were challenged with 10 μg DNP_{38} Ficoll i/p.

‡ Mice were challenged with 100 μg DNP-CGG (alum) i/p.

Table 7. Suppression of the response to DNP-dextran by exposure to DNP-Ficoll

Pretreatment	Challenge*	Direct PFC/spleen	Indirect PFC/spleen
Nil	100 μg DNP-Dextran	51,483 (43,715–60,630)	30,429 (21,161–43,754)
1 mg DNP_{38} Ficoll	100 μg DNP-Dextran	7776 (6776–8925)	5109 (4338–6016)

* Mice were challenged 7 days after pretreatment.

Table 8. Effect of adjuvant in the thymus-independent challenge on tolerance induced by DNP-Ficoll

Pretreatment	Challenge	Direct PFC/spleen	Indirect PFC/spleen
Nil	10 μg DNP_{38}Ficoll + *B. pertussis*	42,315 (33,417–53,584)	19,387 (15,741–23,877)
1 mg DNP_{14} Ficoll	10 μg DNP_{38}Ficoll + *B. pertussis*	2941 (1898–4557)	2164 (1589–2946)

* Mice were challenged 7 days after pretreatment.

Table 9. *In vitro* tolerance induction in primed and unprimed cells by DNP-Ficoll

		Thymus independent response*		Thymus dependent response†		
		DNP PFC/spleen		DNP PFC/spleen		CGG PFC/spleen
Spleen cells	Cultured with‡	Direct	Indirect	Direct	Indirect	Indirect
Normal	Medium	19,648 (15,583–24,772)	21,298 (18,290–24,800)	22,002 (14,288–33,881)	59,683 (42,503–83,806)	N.D.
Normal	DNP_{14}Ficoll	6301§ (4733–8309)	4026¶ (2652–6176)	58,118** (43,261–78,078)	134,922†† (92,916–195,917)	N.D.
Primed	Medium	N.D.	N.D.	9755 (8297–11,469)	458,286 (410,576–511,540)	222,745 (206,348–240,445)
Primed	DNP_{14}Ficoll	N.D.	N.D.	4673‡‡ (3871–5640)	40,958§§ (35,614–47,103)	226,382¶¶ (211,509–242,301)

* Challenged with 50 μg DNP_{38} Ficoll. PFC assay day 6.

† Normal cells challenged with 200 μg DNP CGG (alum+ *B. pertussis*) i/p. PFC assay day 8
Primed cells challenged with 15 μg DNP CGG in saline i/v. PFC assay day 6.

‡ Cells were cultured for 48 h under the conditions described in Table 3 in the presence or absence of 250 μg/ml DNP_{14}Ficoll. 4×10^7 primed cells were transferred to irradiated syngeneic (BALB/c × CBA)F_1 mice.

§ $0{\cdot}01 > P > 0{\cdot}005$; ¶ $0{\cdot}005 > P > 0{\cdot}0025$; ** $0{\cdot}1 > P > 0{\cdot}05$; †† $0{\cdot}1 > P > 0{\cdot}05$ ‡‡ $0{\cdot}01 > P > 0{\cdot}005$; §§ $0{\cdot}0005 > P$; ¶¶ $0{\cdot}45 > P > 0{\cdot}40$.

between 20,000 and 60,000 direct PFC/spleen 5 days after immunization. The ability of these conjugates to induce tolerance when given in large doses was investigated. (BALB/c × C57B1)F1 mice were given 250 μg DNP-SIII and challenged 7 days later with either DNP_{38} Ficoll or DNP-CGG. Table 6 shows that both responses are highly significantly suppressed by pretreatment with DNP-SIII. By contrast, animals pre-injected with 1 mg DNP_{38} Ficoll and subsequently challenged with 10 μg DNP_{38} Ficoll or DNP-CGG showed reduced responsiveness only to DNP_{38} Ficoll (Table 6). Analogous results were obtained if a heterologous carbohydrate antigen, namely DNP-dextran, was used as the thymus-independent readout (Table 7). Another possible reason for the inability to suppress the thymus-dependent response could be the presence of adjuvant in this type of challenge. Therefore, (BALB/c × C57B1)F1 mice were tolerized by administration of 1 mg DNP_{14} Ficoll and challenged 7 days later with 10 μg DNP_{38} Ficoll plus 2×10^9 *B. pertussis* organisms as adjuvant. The tolerized mice gave supressed responses despite the presence of adjuvant (Table 8). Thus, pre-immunization with DNP-Ficoll conjugates, at large doses, also seems to highlight a functional difference between thymus-dependent and thymus-independent responses.

These results are seemingly at variance with those reported by Lewis *et al.* (1976) and Desmayard, Pearce & Feldmann (1976). However, both of these groups were observing tolerance induction in primed cells by thymus-independent conjugates. We therefore compared the susceptibility of primed and unprimed cells to tolerance induction by DNP-Ficoll. Normal spleen cells or spleen cells from animals primed 4 weeks previously with DNP-CGG were cultured in the presence or absence of 250 μg/ml DNP_{14} Ficoll for 48 h. After washing these cells were transferred to lethally irradiated syngeneic recipients and the recipients challenged as shown in Table 9. As can be seen in Table 9 normal cells exposed to DNP-Ficoll *in vitro* showed decreased responsiveness to thymus-independent challenge and somewhat enhanced responsiveness to thymus-dependent challenge. By contrast, primed cells pre-exposed to DNP-Ficoll gave suppressed responses to secondary thymus-dependent challenge. This would seem to equate our results with those of other groups and indicates a similarity in the triggering of thymus-independent B cells and primed thymus-dependent B cells.

DISCUSSION

The results presented here show that it is possible to differentially suppress the responsiveness to thymus-independent antigens whilst leaving responsiveness to the same determinant in thymus-dependent form unaffected. Our experiments also rule out certain possible explanations for this result. The complications caused by the suppressive effect of circulating antibody were ruled out by the use of an adoptive transfer system (Table 3); the tolerance to thymus-independent antigens was not overcome by the inclusion of adjuvant (Table 8). The use of DNP-Ficoll as a thymus-independent test antigen avoided the problem of the intrinsic mitogenicity of certain thymus-independent antigens, as DNP-Ficoll is thought to have minimal activity in this respect (Feldmann, Howard & Desmayard, 1975). If these findings are taken in conjunction with the results reported by other groups, the most attractive explanation is that thymus-dependent and thymus-independent antigens stimulate different B-cell subpopulations and that we are observing the differential tolerance of the B1 (thymus-independent) population.

Other data supporting the B1/B2 hypothesis have mainly been based on physical criteria. Gorczinski & Feldmann (1975) found that the two subpopulations could be separated by velocity sedimentation. The B1 cell is reported to be less sensitive to the effects of the cytotoxic drug azathioprine (Galanaud *et al.*, 1976). Lewis *et al.* (1976) have shown that the thymus-independent B cell lacks the C3 receptor, which is consistent with the work of Arnaiz-Villena *et al.* (1975).

Recently Jennings & Rittenberg (1976) have presented data showing that responses to thymus-dependent and thymus-independent antigens were additive in double challenged cultures. Also, the existence of a substrain of inbred mice, CBA/N, which seem to have a genetic defect in their responsiveness to most thymus-independent antigens provides further circumstantial evidence for the B1/B2 hypothesis (Cohen *et al.*, 1976).

However, our data are seemingly inconsistent with some reports in the literature. Desmayard *et al.* (1976) demonstrate the *in vitro* induction of tolerance to a thymus-dependent antigen using DNP-levan, an antigen very similar to DNP-Ficoll. Lewis *et al.* (1976) show *in vivo* abrogation of a thymus-dependent response using ABA-Ficoll (m-azobenzene-p′-arsonate-3-p-hydroxyphenyl-propionyl-aminoethyl-

carbamyl methyl-Ficoll). It is interesting that both groups were using secondary responses as their thymus-dependent readouts. Our experiments (Table 9) indicate that primed B2 cells are indeed tolerized by exposure to DNP-Ficoll in contrast to the behaviour of unprimed B2 cells. Also preliminary experiments indicate that this tolerance is expressed if the secondary challenge is in soluble or particulate form, thus ruling out the possibility of antigen presentation as an explanation for our results.

The possibility still exists that T-dependent and T-independent antigens in fact stimulate the same B cell, but by different mechanisms, and that our tolerance induction regime differentially inhibits the triggering of this population by the thymus-independent mechanism. This seems unlikely when the evidence based on physical separation is taken into account.

It is interesting, however, to note that the primed B2 cell and the virgin B1 cell share many similar characteristics, mainly concerning the method of triggering. Thymus-independent antigens give responses with rapid kinetics, the peak response to DNP-Ficoll occurring at 4–5 days, in our hands, compared to 7–8 days for primary thymus-dependent responses. Good responses are elicited by small doses of soluble thymus-independent antigen. Both these features are also characteristic of secondary thymus-dependent responses. Klinman, Press & Segal (1973) have suggested that antigen-receptor interactions in secondary responses are functionally multivalent compared to monovalent binding in primary interactions. This possibly reflects a higher cell-surface immunoglobulin density in the primed cells (discussed by Klaus, 1975). The multivalent binding in secondary interactions represents yet another point of similarity as the binding of thymus-independent antigens is functionally multivalent (Feldmann & Basten, 1971). The density of the cell-surface immunoglobulin of B1 cells has yet to be quantified; it would be extremely interesting if they were found to have a high density. By analogy this may explain the similarity in tolerance characteristics between the two populations.

Another possibility is that B1 and B2 cells are different stages in the differentiation of the same cell lineage. A progression from B1→B2 seems unlikely in the light of the anomalous responsiveness of the CBA/N substrain (see above) and the apparent lack in normal mice, of a response to the hapten ABA when presented on a thymus-independent carrier whilst it is possible to show thymus dependent responses, albeit secondary responses, to the same determinant (Lewis et al., 1976). This does not preclude a B2→B1 switch, indeed this has been postulated (Mosier, Scher & Paul, 1976).

Insofar as tolerance mechanisms are concerned we are probably looking at three entirely different processes in this communication. DNP-SIII and DNP-MGG probably induce a reversible receptor blockade (Romano, Lerman & Thorbecke, 1976; Aldo-Benson & Borel, 1976), which is consistent with the immediate onset of the effect (Table 4). DNP-Ficoll probably induces a high zone paralysis of the B1 cell analogous to that observed by Feldmann (1972), which is thought to be irreversible (Diener & Feldmann, 1972). The interesting paradox is that the unprimed B2 cell escapes such paralysis and is not prevented from binding thymus-dependent antigens.

The third mechanism, by which soluble thymus-dependent protein immunogens induce tolerance in B1 cells is still unclear. It might be considered possible that since a B1 cell cannot cooperate with T cells, any thymus-dependent antigen interacting with it would act as if it were non-immunogenic and cause receptor blockade. This seems not to be the case in view of the length of time necessary to establish tolerance (Fig. 1). The T-independence of the phenomenon (Table 5) leads one to consider the role of other accessory cells, such as the macrophage, in the induction of unresponsiveness. It has been suggested that the initial differentiational steps taken by B lymphocytes after antigenic stimulation are relatively thymus-independent and it is only later that T cells play a significant role in the generation of antibody-forming cells (Dutton, 1975; Schimpl & Wecker, 1975). It is therefore a possibility that accessory cells, such as macrophages, play a crucial role in the initial decision taken by the B cell and its interaction with thymus-dependent antigens this decision could be to switch off the cell, i.e. to render the cell unresponsive.

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