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Recruitment of virgin B cells into an immune response is restricted to activation outside lymphoid follicles

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SUMMARY

The site and mechanism of recruitment of short-lived new B cells from the bone marrow into the longer-lived recirculating B-cell pool were studied by using kappa-allotype-distinct congenic rats to construct bone marrow chimeras. Chimeras were immunized with antigen in the form of an antigen–antibody complex that is largely restricted to lymphoid follicles, rapidly localizing to follicular dendritic cells (FDC). This form of antigen, although a potent stimulator of memory B cells, was shown to be a very poor inducer of virgin B-cell responses. This was not due solely to differences in receptor affinity, as complexes in which the hapten epitopes were unmasked also evoked little virgin B-cell response. The inability of virgin B cells to be activated by FDC-bound antigen seems to relate to the fact that they are not part of the recirculating pool and do not migrate through lymphoid follicles; restriction of antigen to a draining lymph node also precluded virgin B-cell activation. The evidence presented suggests that activation of virgin B cells at extrafollicular sites in the spleen (red pulp, marginal zone, outer periarteriolar lymphocytic sheath) is required for both their incorporation into immune responses and into the recirculating pool. These experiments also show that established immune responses are maintained by clones activated soon after immunization and not by continued incorporation of new clones.

INTRODUCTION

Via mechanisms that remain ill-defined, the antigen–antibody complexes that become localized onto the surface of follicular dendritic cells (FDC) are important both in driving the expansion of memory B-cell clones during the germinal centre reaction (Klaus & Humphrey, 1977; Coico, Bhogal & Thorbecke, 1983; Klaus *et al.*, 1980) and regulating long-term secondary antibody responses (Tew & Mandel, 1978; Tew, Phipps & Mandel, 1980). Here the availability of complexed antigen to different B-cell populations (Gray, MacLennan & Lane, 1986; MacLennan & Gray, 1986) is considered in relation to the maintenance and clonal make-up of established responses.

It has been speculated previously that complexed antigen on FDC provides a means of selection of high-affinity mutants during a germinal centre reaction (MacLennan & Gray, 1986), and indeed immunizing with complexes is a very effective way of activating memory B cells (Klaus *et al.*, 1980; Klaus, 1978). While masking of antigenic epitopes in the complex does not appear to be a problem for high-affinity memory B cells, it is not clear if virgin B cells can be activated by antigen in this form. Indeed presentation of antigens in immune complexes might provide a mechanism for selecting only those new B-cell specificities that come up to certain affinity standards. It has been suggested that epitopes become available when complexes dissociate as circulating antibody levels fall (Tew *et al.*, 1980), leading to cyclical antibody production (Rombal & Weigle, 1973; Weigle, 1975). This predicts that new B cells would be incorporated periodically into an established response; however, previous experiments with long-term bone marrow chimeras indicate that established serum responses are maintained by a relatively small number of donor memory clones (Gray *et al.*, 1986).

Using kappa-allotype-marked congenic rats to construct bone marrow chimeras in order to look at responses of virgin and memory B cells (Gray *et al.*, 1986; MacLennan & Gray, 1986), it is apparent that, in the presence of memory populations, activation of newly produced virgin B cells is in general restricted to the first 7–10 days of an immune response. Localization of immune complexes onto FDC starts as antibody levels rise during the second week. We proposed that the waning of virgin B-cell activation was because antigen in follicles on FDC is not available to these cells (Gray *et al.*, 1986; MacLennan & Gray, 1986). To see if this was related to the affinity of receptors or the location of antigen, the response of virgin (host) B cells in bone marrow chimeras immunized with immune complexes has been followed, in which the haptenic determinants were either masked or unmasked. Such complexes are known to localize rapidly onto FDC (Humphrey & Frank, 1967;

Abbreviations: BSA, bovine serum albumin; DNP, dinitrophenyl; FDC, follicular dendritic cells; MSH, *Maia squinada* haemocyanin; OVA, ovalbumin; OX, 2-phenyloxazolone; PFC, plaque-forming cells; TDL, thoracic duct lymphocytes.

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Van Rooijen, 1972) in follicles of secondary lymphoid tissues, structures that contain B cells of the recirculating pool (Nieuwenhuis & Ford, 1976; Gray *et al.*, 1982). These and further experiments suggest that the activation of virgin B cells occurs outside follicles and that they require this signal before they can enter the 'mature' recirculating pool.

MATERIALS AND METHODS

Animals

PVG/Ciu rats and the PVG-RI-a^a (DA) κ-allotype-distinct congenic line (Hunt & Fowler, 1981) were bred and maintained in the Institute for Biomedical Research, Füllinsdorf. All rats were between 10 and 16 weeks of age at the start of experiments.

Bone marrow chimeras

Chimeras were constructed as described previously (Gray *et al.*, 1986; Lane *et al.*, 1986) with the aim that almost immediately after reconstitution the host bone marrow exports Kla virgin B cells into a peripheral pool of mature Klb B cells, that includes primed memory populations. Briefly this involves irradiating Kla-recipient rats while shielding their hind limbs with lead blocks (8 Gy γ-irradiation from a Gamma Cell 40 ^{137}Cs source; Atomic Energy Ltd, Canada). One day later these rats were reconstituted with 2×10^8 thoracic duct lymphocytes (TDL) from Klb donor rats primed with dinitrophenylated spider crab (*Maia squinada*) haemocyanin (DNP-MSH) (kindly provided by Professor J. H. Humphrey, Royal Postgraduate Medical School, Hammersmith, London) plus ovalbumin (OVA; Sigma, St Louis, MO). Donor rats were primed with 50 μg DNP-MSH + 50 μg OVA (alum precipitate + *B. pertussis*, Calbiochem, AG Luzern i.p.) and then boosted 1 month later with soluble antigen. TDL was obtained by cannulating 1–2 months after boosting. The validation of this experimental protocol is contained in Gray *et al.* (1986) and Lane *et al.* (1986). Hind limb shielding is used in preference to syngeneic bone marrow reconstitution because in the latter situation very few B cells are exported into the periphery for the first 3 weeks.

Antigen–antibody complexes

Antigens used to form complexes were DNP-MSH (substitution ration 230:1) and OX-MSH (2-phenol-oxazolone, Sigma, substitution ratio 170:1) prepared by standard methods (Gray *et al.*, 1986; Mäkelä *et al.*, 1978). Standard anti-DNP or anti-OX antisera were obtained by immunizing rats with 50 μg alum-precipitated antigen + *B.pertussis* (i.p.) followed by two boosts with 50 μg of soluble antigen. Immune complexes in which hapten epitopes were masked were formed in slight antibody excess to leave few DNP epitopes unbound. The volume of anti-DNP serum used was determined empirically by reacting various volumes of serum with 10 μg DNP-MSH to find the appropriate volume which leaves only residual antibody activity (assessed by radioimmunoassay). After incubation for 1 hr at room temperature, these complexes were injected directly without further treatment. Complexes in which haptenic epitopes were not masked were made in a similar way: 10 μg OX-MSH were reacted with anti-DNP-MSH or DNP-MSH was reacted with anti-OX-MSH. Again complexes were formed in slight antibody excess such that residual anti-carrier (MSH) activity was retained. Absorption of anti-OX antiserum with DNP-MSH did not alter the titre of anti-OX antibodies and likewise OX-MSH absorption did not alter the anti-DNP titre in an anti-DNP antisera, indicating little cross-reaction between haptens.

Immunization

All complexes were injected intravenously such that each animal received 10 μg of antigen. Immunization was 4 days after reconstitution with TDL. This protocol was shown to result in rapid localization (within 24 hr) into follicles by injecting complexes containing fluorescein (FITC)-labelled MSH. To restrict antigen to draining lymph nodes, 5 μg of DNP-MSH were mixed with Freund's incomplete adjuvant and injected into the front footpad. These animals, as well as those immunized with DNP-MSH anti-DNP complexes, were immunized at the same time (i.v.) with 10 μg of soluble OX-OVA. This was an internal control to establish that each chimera was able to make both donor and host responses to this form of antigen.

Detection of donor and recipient antibody responses

Serum anti-DNP and anti-OX responses were measured in a solid-phase radioimmunoassay. DNP-BSA (bovine serum albumin; Sigma) and OX-BSA were coated onto polystyrene plates at 10 μg/ml (50 μl) in 0·1 M carbonate buffer, pH 9·6, at 4° overnight and then plates were blocked with 100 μl 1% BSA in PBS for 30 min at room temperature. Kla (host) and Klb (donor) serum antibody responses were detected using the monoclonal antibodies MARK-3 (Inotech AG, Wohlen) and FH8, respectively. The radioimmunoassay and the standardization required for comparison of Kla and Klb antibody titres in chimeras is described in some detail elsewhere (Gray *et al.*, 1986).

Plaque-forming cell assay

Enumeration of cells producing antibodies to DNP in spleen and lymph nodes was carried out using a direct plaque assay similar to those described elsewhere (Rittenberg & Pratt 1969; Andersson *et al.*, 1973). Sheep red cells were coupled with 30 mg trinitrobenzene sulphonic acid per ml of packed cells. Cell suspensions were made 7 days after immunization and assayed directly (6×10^4 cells per plate; in triplicate) using an appropriate dilution of rabbit complement.

Enumeration of donor and host κ-allotype-bearing cells

The proportions of host and donor B cells were assessed in cell suspensions of spleen and lymph nodes by double immunofluorescence as follows: Klb-bearing cells were detected using biotinylated FH8 followed by FITC-Avidin (Inotech AG). Kla-bearing cells were detected using the MARK-3 mouse monoclonal antibody, which in turn was detected with a rat anti-mouse immunoglobulin conjugated to FITC (Jackson ImmunoResearch/Dianova, Hamburg, FRG). Both preparations included in the final staining step rhodamine-labelled mouse anti-rat immunoglobulin (Jackson ImmunoResearch/Dianova) to enumerate the total B-cell number. Results are expressed as the host or donor allotype cells as a percentage of total B cells.

RESULTS

Responses to DNP-MSH complexed with antibodies to hapten and carrier

In all experiments described in this paper, bone marrow chimeras were constructed in such a way that soon after

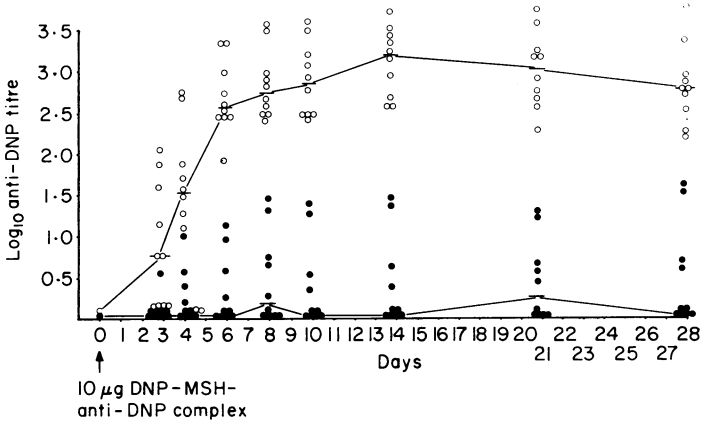

Figure 1. Anti-DNP antibody production of donor (○) and host (●) origin in chimeric rats following immunization with 10 μg DNP-MSH complexed with antiserum to DNP-MSH. Donors were primed with DNP-MSH and OVA. Each circle represents the serum anti-DNP titre from one animal. Lines are drawn between median titres.

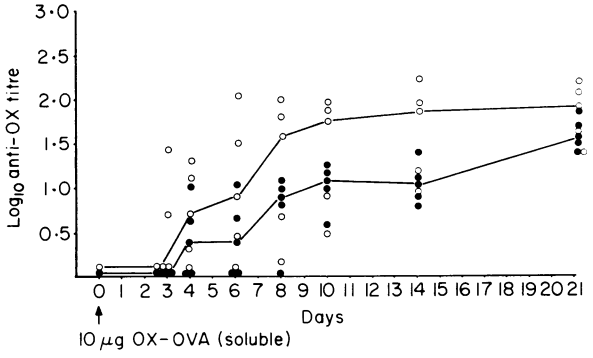

Figure 2. Anti-OX antibody production of donor (○) and host (●) origin in chimeric rats immunized i.v. with 10 μg soluble OX-OVA at the same time as receiving DNP-MSH anti-DNP complex. Donor rats were primed with DNP-MSH and OVA. The five chimeras analysed here are ones that made no host anti-DNP response in Fig. 1. Each circle represents the serum anti-OX titre from one animal. Lines are drawn between median titres.

reconstitution the marrow produces and exports new B cells into peripheral lymphoid tissues that contain recirculating B cells derived from kappa-allotype-distinct hapten-carrier primed donors (see the Materials and Methods). Chimeras were immunized 4 days after reconstitution with TDL and the relative amounts of host and donor serum antibody levels determined over a 28-day period. Hence, antibodies from host or donor origin result, respectively, from stimulation of new, virgin B cells or mature, 'memory' B cells (Gray *et al.*, 1986; Lane *et al.*, 1986).

The results of immunizing chimeras with complexes containing antibodies to both hapten and carrier are shown in Fig. 1. The majority of chimeras (six out of 10) make no host (virgin B-cell) anti-DNP response. Of the four animals that do make virgin (host) B-cell responses, only two maintain serum titres of greater than 1 in 10 for any length of time. This is between 50- and 100-fold lower than donor responses. In contrast to the poor host responses, immunization with these complexes elicits a very potent donor (memory) response.

To ensure that the lack of host response was not due to any trivial problem, such as variable irradiation of the hind limbs, animals were also immunized with soluble OX-OVA (donors were primed with OVA). All chimeras made a host anti-OX response. The response of five of the chimeras that made no host anti-DNP response is shown in Fig. 2. All animals make a host response to oxazolone that reaches similar levels to the donor response within 21 days.

Responses to OX-MSH complexed with antibodies to the carrier only

The poor host (virgin) response to complexes in which the hapten epitope is masked may only reflect the fact that the primed donor population competes more efficiently for available epitopes because of higher affinity receptors. If this is the only reason that virgin B cells are not activated, then complexes that contain antibodies to the carrier only should induce a host response in these chimeras. The result of this experiment is shown in Fig. 3. Donor populations, despite coming from rats not primed with oxazolone, make the majority of the serum antibody throughout the response. The host response during the first 10 days is negligible, rising thereafter to reach a plateau level by 21 days. The same chimeras were also immunized simultaneously with 10 μg soluble DNP-OVA and gave host and donor responses similar to those shown in Fig. 2.

Can the relative amounts of donor and host antibody produced be explained by the relative frequency of host and donor B cells in peripheral lymphoid tissues?

It is not strictly correct to compare amounts of host and donor antibody elicited by a soluble antigen with that evoked by complexed antigen when the oxazolone is on different carriers. Thus Fig. 4 shows the response of host and donor cells to soluble OX-MSH in chimeras in which the donor cells were primed to MSH only. Table 1 shows the ratio of donor to host anti-OX antibody levels in each of these responses both at Day 7 and Day 10. The ratio of donor to host serum antibody in the response to soluble antigen varies between 4:1 and 5:1. This can be explained entirely by the degree of chimerism in peripheral lymphoid organs of these rats; at Day 7 between 20% and 40% of the cells are of host origin (Table 1). However, the ratio of donor to host antibody following immunization with OX-MSH anti-MSH complex is over 120:1.

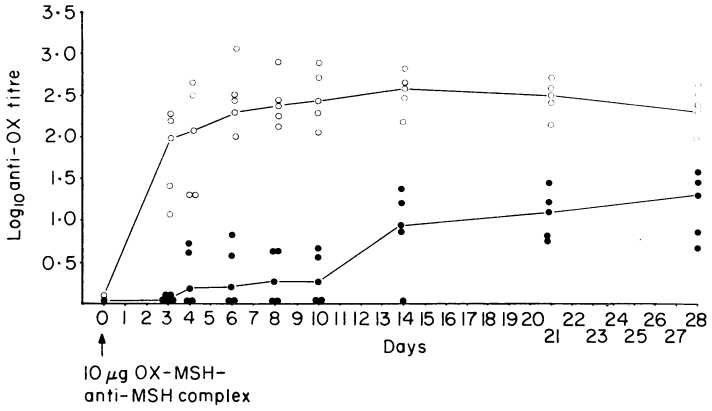

Figure 3. Anti-OX antibody production of donor (○) and host (●) origin in chimeric rats immunized with 10 μg OX-MSH complexed with antisera to MSH, i.e. no antibody to OX epitopes. Donors were primed with DNP-MSH. Each circle represents the serum anti-OX titre from one animal. Lines are drawn between median titres.

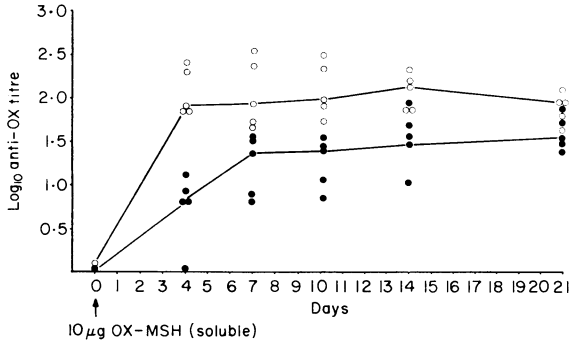

Figure 4. Anti-OX antibody production of donor (○) and host (●) origin in chimeric rats immunized with 10 μg soluble OX-MSH i.v. Donors were primed with DNP-MSH. Each circle represents the serum anti-OX titre from one animal. Lines are drawn between median titres.

Table 1. Chimerism within serum anti-OX responses and within lymphoid tissues

Response to:		Ratios of donor:host anti-OX titre Day 7	Day 10
*OX-OVA (soluble i.v.)	(Fig. 2)	3·9	4·5
*OX-MSH (soluble i.v.)	(Fig. 4)	4·9	5·2
*OX-MSH anti-MSH complex	(Fig. 3)	125·6	126·4
Host allotype cells in chimeric rats at Day 7 (%)			
Lymph node		26·5 (20·1–34·2)†	
Spleen		30·5 (25·5–35·9)	

*Donor cells from rats primed with carrier only.
†Median (range) of five chimeras.

Table 2. PFC response in spleen and draining axillary lymph node following footpad injection

*Dose of DNP-MSH (μg)	Anti-TNP PFC/10^6 input cells: Draining lymph node	Spleen
0·5	6070±3293	5·0±2·2
5·0	9983±3604	9·0±4·5
50·0	22593±11666	419±76
Saline	23±15	12±6·1

*All antigen was given in incomplete Freund's adjuvant.

Can virgin B cells be activated by antigen restricted to a peripheral lymph node?

Virgin B cells are not activated in large numbers by antigen in follicles (on FDC), even when epitopes are free. To test whether the failure of new B cells to migrate to these areas is because they are not part of the recirculating B-cell pool, chimeras were immunized with antigen in a form and dose that was restricted to a draining lymph node and did not reach the spleen. Table 2 shows a dose–response experiment in which the plaque-forming response is looked at in the draining (axillary) lymph node and in the spleen 7 days after a footpad injection of DNP-MSH in Freund's incomplete adjuvant. All doses gave good responses within the lymph node. Only the 50 μg dose showed a small response in the spleen. A dose of 5 μg of DNP-MSH in adjuvant was used in the experiment shown in Fig. 5. The majority of chimeras (six out of nine) make no host (virgin B-cell) response. Three, however, did make a host response, only one of which maintains it to 21 days. This immunization protocol resulted in very effective activation of primed donor cells (the majority of which are part of the recirculating pool being derived from the thoracic duct). All animals responded by producing host antibody to oxazolone following i.v. immunization with soluble OX-OVA.

Do host responses increase with time, without further immunization?

To see whether virgin B cells were recruited into established secondary responses we followed the host responses past Day 28. Ten chimeras from previous experiments (Figs 1 and 5) were chosen, all of whose host response had fallen to zero by 28 days. These fell into two groups: five animals had made a primary host response to hapten while the other five had made no initial host response. Figure 6 shows the serum anti-hapten titres at 52 days. The chimeras that made no initial host response to hapten

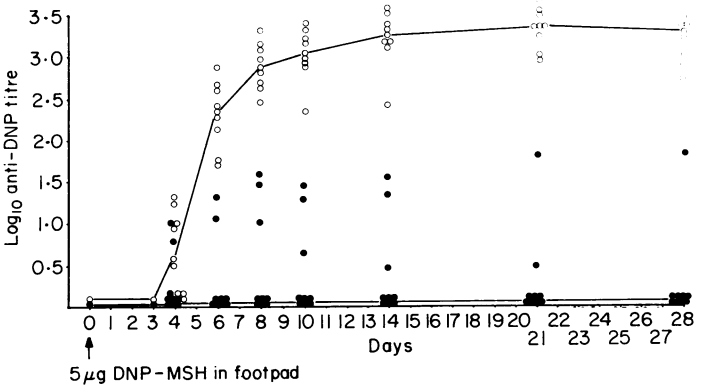

Figure 5. Anti-DNP antibody production of donor (○) and host (●) origin in chimeric rats immunized with 5 μg DNP-MSH in Freund's incomplete adjuvant in the front foot pad. Each circle represents the serum anti-DNP titre from one animal. Lines are drawn between median titres.

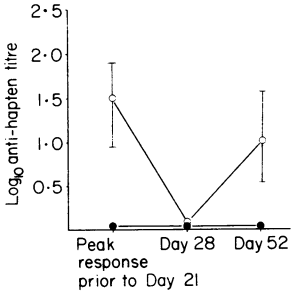

Figure 6. Anti-DNP antibody production of host origin 1 and 2 months after immunization. Chimeras falling into two groups are shown: those that made an initial host response that fell to zero by 28 days (○) and those that made no initial host response (●). Median titres (± ranges) are shown.

maintain their lack of serum antibody over the 2-month period. In contrast chimeras that made a host response within the first month, but whose antibody levels fall to zero by 28 days, did increase their serum anti-hapten titre by Day 52. All chimeras exhibit donor serum anti-hapten titres of greater than 2·5 at both Days 28 and 52.

DISCUSSION

The results presented here show clearly that virgin B cells are not activated effectively by antigen that is complexed with antibody. This is not entirely due to the low affinity of their receptors in competing for antigenic sites that may be masked. Complexes in which hapten epitopes are free are also poor inducers of virgin B-cell responses. It seems probable that virgin B cells do not migrate through the areas in which immune complexes localize. It is well documented that antigen–antibody complexes rapidly localize onto the surface of FDC within follicles (Humphrey & Frank, 1967; Van Rooijen, 1972). Recently Lortan *et al.* (1987) have shown, using cell transfers between κ-allotype distinct congenic rats, that bone marrow B cells (depleted of mature recirculating cells) home very poorly into follicular structures of secondary lymphoid tissues in contrast to thoracic duct lymphocytes (TDL).

That new B cells emerging from the marrow do not immediately go into the recirculating pool is not a new finding: Osmond and co-workers (Brahim & Osmond, 1970, 1976; Yoshida & Osmond, 1978) showed several years ago that bone marrow B cells labelled *in situ* appear in lymph nodes 1–2 days later than they are observed in the spleen. We demonstrate here that by restricting antigen to a lymph node a virgin B-cell response is inhibited. It seems likely that the activation of virgin B cells takes place in the red pulp, the marginal zone or outer PALS of the spleen (Lortan *et al.*, 1987).

As discussed in detail elsewhere (Gray, 1988), this activation is the basis of selection of new B cells for entry into the recirculating pool. Because the recirculating B-cell pool is relatively slow to turn over [average lifespan of a recirculating B cell $\simeq$4 weeks (Gray, 1988; Sprent & Basten, 1973)] it is clear that the 5×10^8 new B cells produced from the bone marrow of a rat every day (Deenen, Hunt & Opstelten, 1987) must undergo a selection process before entry. The data presented here add to the evidence (Gray *et al.*, 1986; MacLennan & Gray, 1986) that antigenic activation is a major mechanism for selection. Chimeras that make an initial transient host anti-hapten response (falling to zero by Day 28) increase their serum antibody titre at later times without further immunization. In contrast, those that made no initial host response will only make antibody after boosting. It seems likely that the virgin B cells recruited into the recirculating pool during the initial phase of the response are reactivated by the long-term depots of antigen on FDC (Tew & Mandel, 1978; Tew *et al.*, 1980). In chimeras that make no initial host response, although antigen is available on FDC (donor responses are maintained over long periods), no virgin B cells are incorporated into this established response. This would seem to rule out the idea that immune complex on FDC acts as a screen for 'high-affinity' new B cells (Tew *et al.*, 1980). As we suspected previously (Gray *et al.*, 1986), established secondary responses are maintained by memory clones with little or no incorporation of new clones.

We do not address here the events that lead to this clonal dominance other than to assert that initially they occur outside follicles. However, these data do not contradict the finding that B cells in naive animals are primed very effectively by antigen–antibody complexes (Klaus, 1978). In those experiments immu-

nization with complexes enhances memory IgG responses but induces very poor primary responses. While the enhanced memory B-cell expansion is certainly due to rapid localization to FDC, it is likely that the primary stimulus to these cells was given by complexes taken up by presenting cells at extrafollicular sites (Tew & Mandel, 1979; Mandel *et al.*, 1980).

While we have attributed the lack of virgin B-cell responses to antigen relocalization to follicles following administration of immune complexes, other interpretations should be considered. Firstly, it is well known that administration of relatively large amounts of free IgG antibody leads to an inhibition of primary responses (Dennert, 1971). The complexes used here are made in slight antibody excess and so the amount of free antibody transferred is small. It is also clear that such IgG-mediated suppression is antigen specific (Heyman & Wigzell, 1984; Caulfield & Shaffer, 1987); the crucial experiment reported here is one in which the complex contains no antibody (free or otherwise) that binds to the hapten and still the virgin B-cell response to this hapten is poor. Secondly, the phenomenon of hapten-specific suppression (Herzenberg, Tokuhisa & Herzenberg, 1980) is an unlikely explanation for these results as in all chimeras both donor and host cells respond to soluble OX-OVA after carrier priming of the donor cells.

We found few groups, immunized with complexes, in which host responses were entirely absent; in other words in some animals host (virgin) B cells are activated. A possible reason for this is that, following injection, complexes are taken up by macrophages or dendritic cells and presented to B cells at extrafollicular sites. Certainly only a fraction of the injected complex localizes onto FDC, the rest is phagocytosed and degraded by macrophages (Tew & Mandel, 1979; Mandel *et al.*, 1980). In an attempt to avoid this problem experiments were repeated by prelocalizing complexes onto FDC of recipient rats 5 days before irradiation. The results (not shown) were very similar to those shown in Figs 1 and 3. Thus we should be aware that the activation of virgin B cells by antigen may not be the sole mechanism of recruitment into the recirculating pool. An antigen-independent pathway may operate, as it certainly does during the repopulation of peripheral B-cell populations that have been depleted by anti-μ and anti-δ treatment from birth (Bazin *et al.*, 1985).

Such a pathway would explain why chimeras immunized with OX-MSH anti-MSH complexes make host antibodies only after 10 days (Fig. 3). Virgin B cells recruited non-specifically into the recirculating pool might be activated by unmasked hapten epitopes on FDC. The lack of virgin B-cell incorporation during normal established responses (Fig. 6) is probably due to masking of hapten epitopes. That donor anti-hapten responses in Figs 2, 3 and 4 originate from thoracic duct populations that received no priming with hapten also indicates the existence of an antigen-independent pathway. However, whether the selection is really non-specific depends on the affinity-threshold of the interactions involved. In other words, a positive signal may be delivered by cross-reacting antigens.

In intact animals, as only $\sim 10\%$ of the new B cells exported become stably incorporated into the recirculating pool (Gray, 1988), the selection process might be expected to relate to a functional property of the cells. It is possible that a selection process based on antigenic activation may only require low-affinity interactions. It seems disadvantageous at the start of an immune response to discard even very low-affinity clones when somatic mutation of 'low-affinity' V genes could produce a high-affinity antibody after only a few changes; just as a few changes might abolish antigen binding of a high-affinity antibody. It has been shown recently that low-affinity antibodies not normally detected by hybridoma supernate screening are involved in the early response to oxazolone (Pelkonen, Kaartinen & Mäkelä, 1986; Kaartinen, Pelkonen & Mäkelä, 1986). We have data (unpublished) that in the oxazolone response such low-affinity clones expressing V_HOX1/V_KOX1-related genes (Kaartinen *et al.*, 1983) do become part of a germinal center reaction.

The data presented here indicate that virgin B-cell activation occurs at extra-follicular sites. Because this only takes place during the first 2 weeks of the response (Gray *et al.*, 1986; MacLennan & Gray, 1986), it is during this time that the clonal character of the response is established. It changes thereafter only due to somatic mutation and loss of memory clones through competition but not by further recruitment of virgin B cells.

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REFERENCES

Andersson J., Melchers F., Galanos C. & Lüderitz O. (1973) The mitogenic effect of lipopolysaccharide in bone marrow-derived mouse lymphocytes. Lipid A as the mitogenic part of the molecule. *J. exp. Med.* **137,** 943.

Bazin H., Platteau B., MacLennan I.C.M. & Johnson G.D. (1985) B cell production and differentiation in adult rats. *Immunology*, **54,** 79.

Brahim F. & Osmond D.G. (1970) Migration of bone marrow lymphocytes demonstrated by selective bone marrow labelling with thymidine-^{3}H. *Anat. Rec.* **168,** 139.

Brahim F. & Osmond D.G. (1976) Migration of newly-formed small lymphocytes from bone marrow to lymph nodes during primary immune responses. *Clin. exp. Immunol.* **24,** 515.

Caulfield M.J. & Shaffer D. (1987) Immunoregulation by antigen/antibody complexes. I. Specific immunosuppression induced *in vivo* with immune complexes formed in antibody excess. *J. Immunol.* **138,** 3680.

Coico R.F., Bhogal B.S. & Thorbecke G.J. (1983) Relationship of germinal centers in lymphoid tissue to immunologic memory. VI. Transfer of B cell memory with lymph node cells fractionated according to their receptors for peanut agglutinin. *J. Immunol.* **131,** 2254.

Deenen G.J., Hunt S.V. & Opstelten D. (1987) A stathmokinetic study of B lymphopoiesis in rat bone marrow: proliferation of cells containing cytoplasmic μ-chains, terminal deoxynucleotidyl transferase and carrying HIS 24 antigen. *J. Immunol.* **139,** 702.

Dennert G. (1971) The mechanism of antibody induced stimulation and inhibition of the immune response. *J. Immunol.* **106,** 951.

Gray D. (1988) Population kinetics of rat peripheral B cells. *J. exp. Med.* **167,** 805.

Gray D., MacLennan I.C.M., Bazin H. & Khan M. (1982) Migrant $\mu^+\delta^+$ and static $\mu^+\delta^-$ B lymphocyte subsets. *Eur. J. Immunol.* **12,** 564.

Gray D., MacLennan I.C.M. & Lane P.J.L. (1986) Virgin B cell recruitment and the lifespan of memory clones during antibody

responses to 2, 4-dinitrophenyl-hemocyanin. *Eur. J. Immunol.* **16,** 641.

HERZENBERG L.A., TOKUHISA T. & HERZENBERG L.A. (1980) Carrier-priming leads to hapten-specific suppression. *Nature (Lond),* **285,** 664.

HEYMAN B. & WIGZELL H (1984) Immunoregulation by monoclonal sheep erythrocyte-specific IgG antibodies: suppression is correlated to level of antigen binding and not to isotype. *J. Imunol.* **132,** 1136.

HUMPHREY J.H. & FRANK M.M. (1967) Localisation of non-microbial antigens in the draining lymph nodes of tolerant, normal and primed rabbits. *Immunology,* **16,** 87.

HUNT, S.V. & FOWLER M.H. (1981) A repopulation assay for B and T lymphocyte stem cells employing radiation chimeras. *Cell Tissue Kinet.* **14,** 445.

KAARTINEN M., GRIFFITHS G.M., MARKHAM A.F. & MILSTEIN C. (1983) mRNA sequences define an unusually restricted IgG response to 2-phenyloxazolone and its early diversification. *Nature (Lond.),* **304,** 320.

KAARTINEN M., PELKONEN J. & MÄKELÄ O. (1986) Several V genes partipate in the early phenyloazolone response in various combinations. *Eur. J. Immunol.* **16,** 98.

KLAUS G.G.B. (1978) The generation of memory cells. II. Generation of B memory cells with pre-formed antigen–antibody complexes. *Immunology,* **34,** 643.

KLAUS G.G.B & HUMPHREY J.H. (1977) The generation of memory cells. I. The role of C3 in the generation of B memory cells. *Immunology,* **33,** 31.

KLAUS G.G.B., HUMPHREY J.H., KUNKL A. & DONGWORTH D.W. (1980) The follicular dendritic cell: its role in antigen presentation in the generation of immunological memory. *Immunol. Rev.* **53,** 3.

LANE P.J.L., GRAY D., OLDFIELD S. & MACLENNAN I.C.M. (1986) Differences in the recruitment of virgin B cells into antibody responses to thymus-dependent and thymus independent type-2 antigens. *Eur. J. Immunol.* **16,** 1569.

LORTAN J.E., ROOBOTTOM C.A., OLDFIELD S. & MACLENNAN I.C.M. (1987) Newly-produced virgin B cells migrate to secondary lymphoid organs but their capacity to enter follicles is restricted. *Eur. J. Immunol.* **17,** 1311.

MACLENNAN I.C.M. & GRAY D. (1986) Antigen driven selection of virgin and memory B cells. *Immunol. Rev.* **91,** 63.

MÄKELÄ O., KAARTINEN M., PELKONEN J.L.T. & KARJALAINEN K. (1978) Inheritance of antibody specificity. V. Anti-2-phenyloxazolone in the mouse. *J. exp. Med.* **148,** 1644.

MANDEL T.E., PHIPPS R.P., ABBOT A. & TEW J.G. (1980) The follicular dendritic cell: long-term antigen retention during immunity. *Immunol. Rev.* **53,** 29.

NIEUWENHUIS P. & FORD W.L. (1976) Comparative migration of T and B cells in the rat spleen and lymph nodes. *Cell Immunol.* **23,** 254.

PELKONEN J., KAARTINEN M. & MÄKELÄ O. (1986) Quantitative representation of two germ-line V genes in the early antibody response to 2-phenyloxazolone. *Eur. J. Immunol.* **16,** 106.

RITTENBERG M.B. & PRATT K.L. (1969) Anti-trinitrophenyl (TNP) plaque assay. Primary response of BALB/c mice to soluble and particulate immunogen. *Proc. Soc. exp. Biol. Med.* **132,** 575.

ROMBAL C.G. & WEIGLE W.O. (1973) A cyclical appearance of antibody antigen. *J. exp. Med.* **138,** 1426.

SPRENT J. & BASTEN A. (1973) Circulating T and B cells of the mouse. II. Lifespan. *Cell Immunol.* **7,** 40

TEW J.G. & MANDEL T.E. (1978) The maintenance and regulation of serum antibody levels: evidence indicating a role for antigen retained in lymphoid follicles. *J. Immunol.* **120,** 1063.

TEW J.G. & MANDEL T. (1979) Prolonged antigen half-life in the lymphoid follicles of specifically immunised mice. *Immunology,* **37,** 69.

TEW J.G., PHIPPS R.P. & MANDEL T.E. (1980) The maintenance and regulation of the humoral immune response: persisting antigen and the role of follicular antigen-binding dendritic cells as accessory cells. *Immunol. Rev.* **53,** 175.

VAN ROOIJEN N. (1972) Antigens in the spleen. The non-specificity of the follicles in the process of antigen trapping and the role of antibody. *Immunology,* **22,** 757.

WEIGLE W.O. (1975) Cyclical production of antibody as a regulatory mechanism in the immune response. *Adv. Immunol.* **21,** 87.

YOSHIDA Y. & OSMOND D.G. (1978) Homing of bone marrow lymphoid cells: localization and fate on newly-formed cells in lymphocyte-rich marrow fractions injected into lethally irradiated recipients. *Transplantation,* **25,** 246.