

# IMMUNE RESPONSE TO BACTERIAL DEXTRANS

## II. T Cell Control of Antibody Isotypes

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It is established that stimulation of IgM-bearing, immunocompetent B cells, gives rise to progenies expressing the same variable regions in conjunction with a non-IgM heavy chain (1-4). This phenomenon is designated heavy chain class switch and the process is known to require mitosis (5) and DNA recombination (6, 7). Although the organization and structure of the genes in the heavy chain cluster are known (6, 7), the control of class switch is still a matter of debate. It has been argued that the class-switch is a random process (8) and that proliferating B cells necessarily delete C-genes in the direction of transcription, to end up as IgA secretors. Recent reports, however, have shown that the isotype expression in both in vivo and in vitro immune responses is strictly regulated (9, 10). Little is known on the nature of that regulation.

Although both thymus-dependent (TD)<sup>1</sup> and thymus-independent (TI) antigens give rise to plaque-forming cell (PFC) responses of IgM and non-IgM isotypes, there is a marked difference in the isotypes induced by these two types of antigens. Thus, TI antigens primarily induce IgM and IgG3 PFC (4, 11-13), while TD antigens preferentially induce IgM, IgG1, and IgA PFC (14-17). This isotype selectivity is basically carrier-specific, and it has been interpreted to indicate: (a) an inherent ability of different B cell subsets responding to either type of antigen, to switch in a programmed manner (13); (b) the involvement of Ig-specific regulatory cells influencing the class switch of the B cells (14); (c) the direct control of isotype production, by the quality of the stimuli activating the responding B cells to clonal expansion and terminal maturation (18-21).

It has previously been reported that the TI-2 antigen Dextran B512 (Dex), shows a marked strain-dependent ability to induce specific IgG PFC. Thus, C57BL/6 but not CBA mice, produce IgG anti-Dex PFC (22) although both strains are "high" responders to Dex. Since we (unpublished observations) and others (23) have isolated Dex-specific hybridomas of the IgA isotype, it was of interest to reevaluate isotype expression in the Dex-specific response.

In this study we show that a large fraction of the Dex-specific PFC induced in C57BL/6 mice are of IgA isotype, and we report on the involvement of T cells

<sup>1</sup> *Abbreviations used in this paper:* a-Id, antiidiotypic sera; BGG, bovine gamma globulin; BSA bovine serum albumin; Dex, dextran; DNP, dinitrophenyl; ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; PFC, plaque-forming cells; r.t., room temperature; SRC, sheep erythrocytes; TD, thymus dependent; TI, thymus independent; TNP, trinitrophenyl.

in the response of all non-IgM isotypes. We have also investigated the differential isotype patterns induced by Dex as a carrier and as an epitope, respectively. It is suggested that one of the isotype controls participating in Dex responses are idiotype-specific T cells.

### Materials and Methods

*Mice.* C57BL/6 mice were bred in our own facilities. C57BL/6 nu/nu mice were purchased from Bomholtgård, Ry, Denmark.

*Antigens and Immunizations.* Native Dex B512 (mol wt  $10-50 \times 10^6$ ) from *Leuconostoc mesenteroides*, a kind gift of Dr. W. Richter, Pharmacia, Uppsala, Sweden, was injected intravenously (i.v.) at a dose of 10  $\mu\text{g}$ /mouse. Dinitrophenylated Dex (DNP-Dex) was prepared as described (24) by reacting native Dex with *N*, $\epsilon$ -DNP-L-lysine HCl (Sigma Chemical Co., St. Louis, MO) (coupling ratio: 1.3 mol DNP/100 mol glucose). A conjugate of Dex T20 (Pharmacia) to bovine serum albumin (Dex-BSA) was prepared using the same method. DNP-Dex and Dex-BSA were kind gifts of Dr. C. Fernandez, Department of Cell Research, Uppsala, Sweden, and were used for i.v. immunization diluted 1/100 and 1/50, respectively, given in 0.2-ml doses. Dinitrophenylated bovine gamma globulin (DNP<sub>16</sub>-BGG) was prepared according to Little and Eisen (25) and used for i.v. immunization at 50  $\mu\text{g}$ /mouse.

*PFC Assays.* The number of direct Dex-specific PFC in spleen cell suspensions were detected in microscope slide chambers, using Dex-conjugated sheep erythrocytes (Dex-SRC) and guinea pig complement (Lot 44011048 Flow Laboratories, McLean, VA), as previously described (26). Dex-specific PFC of non-IgM isotypes were detected by adding, to the plaque-suspension, class-specific antisera prepared in rabbits against mouse myeloma proteins of the various isotypes (20). In some experiments rabbit anti-total mouse immunoglobulin sera were used to reveal PFC of all non-IgM isotypes. The derivation and characterization of the developing antisera has previously been described elsewhere (20, 27) and they were used at dilutions for maximal plaque-developing activity. Direct trinitrophenyl (TNP)-specific PFC were detected in a variant of the hemolytic plaque assay of Jerne and Nordin (28), using TNP conjugated SRC (TNP<sub>10</sub>-SRC) as described in detail (29). To detect non-IgM anti-TNP PFC, the developing antisera above were included in the plaque suspension, at appropriate dilutions for optimal plaque-developing activity. All figures reported for non-IgM PFC, represent the difference between the number of plaques obtained in the presence of developing antisera and the number of direct PFC. In parallel, the number of SRC-specific PFC were assayed in each experiment and subtracted from these figures.

*Enzyme-linked Immunosorbent Assay (ELISA).* Dex-immunized mice were treated with 10 U of Dextranase (Sigma) intraperitoneally (i.p.) 24 h before bleeding, to degrade circulating Dex. The amounts of Dex-specific antibodies in sera, were determined in ELISA assays, described in detail elsewhere (30). Briefly, wells of polyvinyl microplates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with 10  $\mu\text{g}$  of Dex T 2000 (Pharmacia), dissolved in phosphate-buffered saline (PBS), for 2 h at room temperature (r.t.). After washing with PBS, the wells were saturated with 3% BSA in PBS for 30 min at r.t. and after washing, the test sera were added and incubated for 2 h at r.t. Bound antibodies were detected using class-specific rabbit antisera, the specificity of which has previously been described (20). After washing, peroxidase-conjugated swine anti-rabbit Ig serum (Dacopatts A/S, Denmark) was added and after 2 h at r.t. the wells were washed and exposed to the substrate orthophenylenediamine HCl (Sigma). After stopping the reaction with 10% sodium dodecyl sulfate, the absorbance was recorded at 450 nm using a Titertek Multiscan spectrophotometer (Flow Laboratories). A Dex-specific monoclonal antibody, from the hybridoma D.16. ( $\mu$ ,k), derived from a fusion of Dex-immune C57BL/6 spleen cells and the nonsecreting myeloma Sp 2/0 (D. Holmberg and D. Juy, unpublished observations), was used as specificity control for the class-specific antisera.

*Detection of Idiotypes on PFC.* Antiidiotypic antisera (a-Id) against the C57BL/6 Dex-specific hybridoma 17-9 were prepared as described (31). The specificity of such a-Id has previously been reported (32). To detect Dex-specific IgM or IgA PFC expressing the 17-9 idiotypic, various threefold dilutions of the a-Id were included (15  $\mu$ l/slide) in the plaque-suspension. Nonspecific inhibition of PFC by the a-Id was tested for by including the same amounts of a-Id in assays of SRC-specific PFC from SRC immune ( $5 \times 10^6$  SRC/mouse i.v.) C57BL/6 mice and is subtracted from the reported figures. The nonspecific inhibition did not exceed 10%, neither for IgM nor for IgA PFC. The results are given as percent inhibition of the number of PFC in control slides without a-Id.

## Results

*Kinetics of the Isotype Expression in the Dex-specific Response.* It has previously been described by Fernandez and Möller (22) that mouse strains of the IgCH<sup>b</sup> haplotype produce IgG PFC in the response to Dex, while IgCH<sup>i</sup> strains fail to do so. The production of IgG PFC was reported to be independent of T cells (22). Since IgA-secreting hybridomas can be derived from Dex-immune mice very frequently (reference 23, and our unpublished observations), we decided to investigate in more detail isotype expression in the Dex response of C57BL/6 mice and its control.

In a first set of experiments, the kinetics of the Dex-specific PFC response of all isotypes were studied in C57BL/6 mice. The results of a representative experiment are shown in Fig. 1. In addition to IgM the most represented Ig classes are IgA, IgG3, and IgG2b. The expression of IgG2b and IgG3 isotypes was expected, since both these isotypes have been characterized in the responses to some TI antigens with similar characteristics (9, 11).

In contrast, IgA responses were surprising, as they are known to strictly depend on the participation of helper T cells (33, 34). Both the IgA and IgM Dex-specific PFC responses are maximal on day 5 (Fig. 1), suggesting that B cells activated by Dex switch directly from IgM to IgA secretion, as previously suggested by other experiments (35). Importantly, not all of the Dex-immune

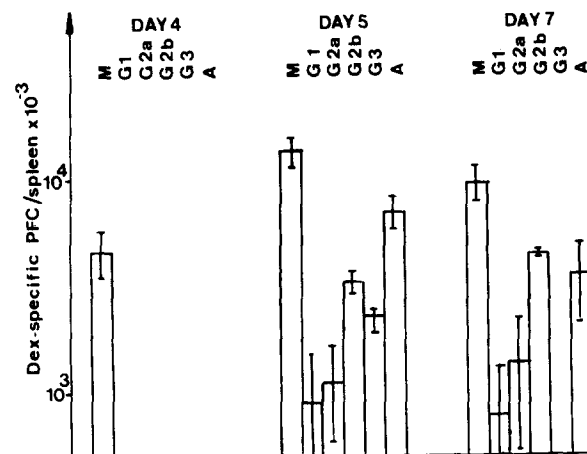


FIGURE 1. Groups of four C57BL/6 mice were immunized with 10  $\mu$ g Dex i.v. and the number of Dex-specific PFC of indicated isotypes assayed on indicated days thereafter. The bars represent geometric means  $\pm$  1 SD of the number of PFC/spleen.

mice assayed produce specific PFC of all the isotypes. Thus, although the mice in these experiments were matched for age and sex, even taken from the same cage, we observed a marked individual variability in this respect. We have previously shown (26) that the overall magnitude of responses to Dex is characterized by individual variability as well.

*Non-IgM Isotype Expression in the Dex Response is T Cell Dependent.* While compatible with similar studies on the response to Dex B 1355 (36), these results suggested that the response to the TI-2 antigen Dex, although largely T cell independent, may be modulated by T cells. Nude and normal euthymic C57BL/6 mice were immunized with Dex and their PFC responses assayed 5 d later. As outlined in Table I, nude mice produce high amounts of IgM PFC as compared with normal euthymic mice, but completely fail to express non-IgM PFC, in contrast with normal littermates. These results are clearly not compatible with those of Fernandez and Möller (22), who reported the T cell-independent production of IgG PFC in the response to Dex. In those experiments, however, lethally irradiated, thymectomized, and bone-marrow reconstituted mice were used, which may explain this discrepancy.

Since other organs for plasma cell localization are well known, we investigated whether or not the isotypes expressed by splenic Dex-specific PFC are representative of the whole immune responses. We have therefore analyzed Dex-

TABLE I  
*Production of Non-IgM Dex-specific PFC Requires Presence of Functional T Cells*

Strain	Dex-specific PFC/spleen	
	Direct PFC (IgM)	Indirect PFC (IgG + IgE + IgA)
C57BL/6 +/+	42,260 (27,595-64,710)*	19,350
C57BL/6 nu/nu	111,052 (87,129-141,546)	<100

All mice were injected with 10 µg Dex i.v. and assayed for Dex specific PFC 5 d later. The figures are geometric means of five mice/group.

\* Range of PFC responses.

TABLE II  
*Expression of Specific Antibody Isotypes in Dex-immune Sera*

Strain	Treatment	Reciprocal of anti-Dex serum titer*					
		IgM	IgG3	IgA	IgG1	IgG2a	IgG2b
C57BL/6 +/+	Dex	7,333 <sup>‡</sup> (5,000-14,000)	58 (<5-140)	158 (<5-400)	<5	<5	<5
C57BL/6 +/+	None	37 (<10-50)	<5	8 (<5-13)	<5	<5	<5
C57BL/6 nu/nu	Dex <sup>‡</sup>	48,000	<5	<5	<5	<5	<5
C57BL/6 nu/nu	None	<5	<5	<5	<5	<5	<5
D.16 <sup>‡</sup>		2.5 ng/ml	>10 µg/ml	>10 µg/ml	>10 µg/ml	500 ng/ml	>10 µg/ml

Euthymic (+/+) or athymic (nu/nu) C57BL/6 mice were immunized with 10 µg Dex i.v. or left untreated. The mice were treated with 10 U of Dextranase i.p. 24 h before bleeding, days 6 and 7 post immunization.

\*The serum dilution giving an OD<sub>450</sub> of 0.05, was extrapolated from titration curves of the Dex-immune sera, and considered as the Dex-specific titer.

<sup>‡</sup>Geometric mean of sera from 6 individual mice, the range is given in parenthesis.

<sup>§</sup>Pool of sera from four individual mice.

<sup>‡</sup>Dex-specific hybridoma antibody (see Materials and Methods).

specific serum antibodies in class-specific ELISA assays. Since Dex persists for a long time in circulation, Dex-immune mice were treated with dextranase (37) 24 h before bleeding, in order to unmask all Dex-specific antibodies in the sera. Table II shows the levels of Dex-specific antibody, of various isotypes, in sera from mice bled days 6 and 7 post injection. As can be seen, Dex-specific antibody of IgM, IgG3, and IgA isotypes can be detected in sera of euthymic Dex-immune mice, whereas in athymic mice IgG3 and IgA antibodies are undetectable. It should be pointed out that Dex-specific antibody of IgG3 and IgA isotypes could not be detected in all sera tested. Thus, also in this respect, the immune response to Dex is characterized by a profound individual variability, as was found for PFC responses (Fig. 1) and for the overall responsiveness to Dex (26). In contrast to the data obtained in the plaque assay, however, only IgA and IgG3 antibodies are detected in sera from euthymic mice in this assay system, and no IgG1, IgG2a, and IgG2b could be found. There are several explanations for this discrepancy. Thus, the low affinity of Dex-specific antibodies (38) may result in too-high threshold levels of concentration, for detection of Dex-specific IgG antibodies in serum. Furthermore, plasma cells producing Dex-specific antibody of IgM, IgG3, and IgA isotypes might be localized in lymphoid organs other than spleen, which would explain why these isotypes are present in sufficiently high amounts to be detected. We find it very unlikely that the Dex-specific IgG PFC detected would be due to artifacts of the developing antisera used, since one would then expect that indirect PFC would be detectable in nude mice as well.

At any rate, these experiments clearly demonstrate an unusual isotype pattern (IgM, IgA, IgG3) and the T cell modulation of such isotype expression.

*Dex-induced T Cell Activities Discriminate between Dex and DNP-specific B Cells.* These regulatory T cells demonstrated above could either be Dex-specific or recognize the responding B cells themselves, i.e. be antiidiotypic. In the first case, one would expect that if Dex is used as a carrier for another epitope (DNP) similar patterns of isotype expression would be obtained in the response to the Dex-linked hapten. In the second case, isotype patterns would be quite different, due to differences in variable region determinants between Dex- and DNP-specific B cells.

C57BL/6 mice were immunized with optimal doses of either DNP-Dex or Dex-BSA and assayed for DNP and/or Dex-specific PFC 5 d later. The response to the TD form of Dex (Dex-BSA) leads to expression of the more "T cell-dependent" isotypes IgG1 and IgG2a in addition to the classes detected in response to Dex itself, i.e. IgA, IgG3, and IgG2b (Table III and Fig. 2). It would appear that there are two threshold levels of T cell participation in the response to Dex-BSA, namely a higher level of protein carrier-specific help inducing IgG1 and IgG2a, and a lower level of Dex carrier-specific help leading to IgG3 production. In addition, a distinct type of T cell help or modulation, which acts on the Dex-specific B cells directly, appears to lead to IgA production.

The response to DNP-Dex confirmed these indications, as the patterns of isotypes expressed by DNP-specific PFC are quite different from those of Dex-specific PFC induced by either Dex or Dex-BSA. High levels of IgG3 PFC are

TABLE III  
*Dex-induced T Cell Activities Discriminate Between Dex and DNP-specific B Cells\**

Carrier	Dex-specific PFC/spleen					TNP-specific PFC/spleen					
	IgM	IgG1	IgG2a	IgG2b	IgG3	IgM	IgG1	IgG2a	IgG2b	IgG3	IgA
Dex	14,300 ± 6,469	1,933 ± 1,850	2,000 ± 2,864	3,433 ± 1,069	2,233 ± 702	62,220 ± 7,804	<500	798 ± 310	<500	5,380 ± 3,046	<500
Proteins <sup>‡</sup>	9,064 ± 1,350	1,871 ± 420	2,233 ± 843	1,379 ± 477	3,377 ± 859	3,166 ± 633	928 ± 752	3,805 ± 1,072	7,056 ± 2,078	<500	<500

C57BL/6 mice were immunized with Dex, Dex-BSA, DNP-Dex or DNP-BGG i.v. (see Materials and Methods) and assayed for Dex- and/or TNP-specific PFC 5 d later. The figures are geometric means ± 1 SD of eight mice/group.

\* Data taken from Fig. 1.

‡ BSA-Dex and BGG-TNP for Dex-specific and TNP-specific responses, respectively.

## CONTROL OF ISOTYPE EXPRESSION

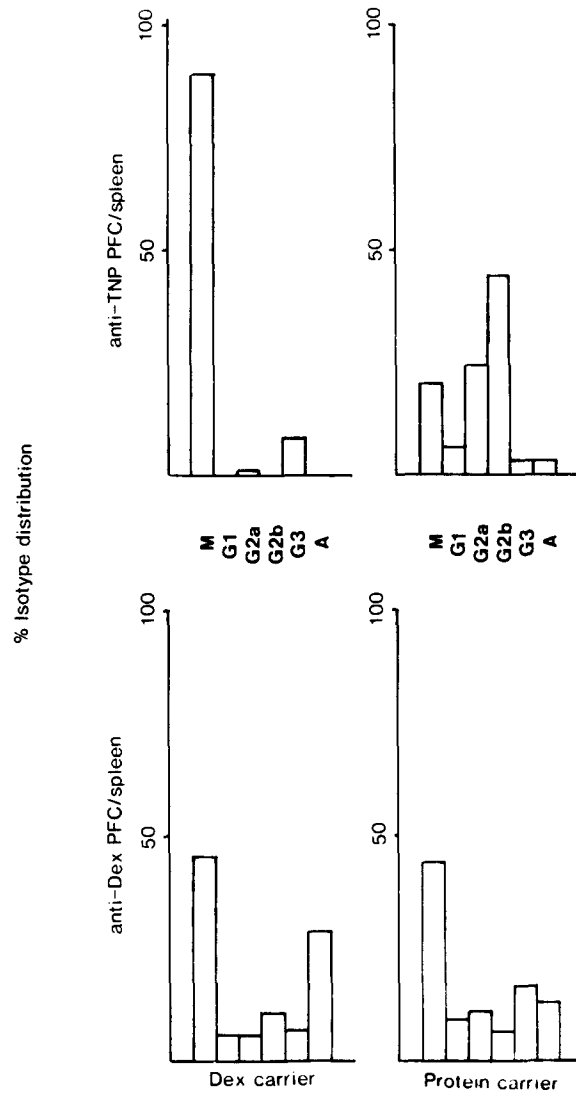


FIGURE 2. The same results shown in Table III are plotted as percent distribution of antigen-specific PFC in the various isotypes.

induced, but the IgA response is completely absent. We conclude that the T cell activities induced by Dex in this response do not influence the isotype expression of DNP-specific B cells in the same manner, although DNP-Dex was prepared from native Dex and thus, would display the same carrier properties. The fact that the TD conjugate DNP-BGG did not induce IgA PFC either, reinforces the epitope-specificity of the IgA response as it would be very unlikely that DNP-specific B cells are functionally defective in switching to IgA secretion. On the other hand, the isotypes induced in *in vivo* immune responses are very dependent on parameters such as dose of antigen and route of immunization (16, 39) and it may be that the low expression of IgA DNP-specific PFC in the responses to

both DNP-BGG and DNP-Dex is peculiar to the immunization regimen used here. In contrast to the isotype pattern observed by Mongini et. al. (40, 41) in response to TNP conjugates of the TI-2 antigens Ficoll and Levan, both in vivo and in vitro, we did not observe detectable amounts of IgG1 and IgG2b TNP-specific PFC responses to DNP-Dex.

*Dominant Idiotype Expression by Dex-specific IgA PFC.* If the T cells involved in the response to Dex were isotype-specific, one would not expect that the expression of non-IgM PFC would show the same kinetics as the IgM Dex-specific response (Fig. 1), in particular since the background levels of Dex-specific PFC, are very low in normal mice, both in spleen and bone-marrow (not shown). Most important, isotype-specific helper activity could not explain the differential isotype patterns of Dex- or TNP-specific PFC after immunization with Dex carrier. It seems unlikely therefore, that isotype-specific T cells would have a major role in the Dex-response. The alternative for the specificity of the T cells participating in the production of IgA in Dex-specific responses is that such cells are idiotype-specific. We have, in fact, previously shown the existence of helper T cells in normal, unimmunized C57BL/6 mice (32), with specificity for a "recurrent" idiotype expressed by a Dex-specific hybridoma antibody derived from the same strain. This model, however, would require that similar idiotypes are dominantly expressed both in the IgM and the IgA classes of anti-Dex PFC. Table IV shows that this is indeed the case. Thus, syngeneic antiidiotypic antibodies prepared against the Dex-specific C57BL/6 hybridoma antibody 17-9, which expresses an idiotype cross-reactive with the previously described 38-13 antibody (32), inhibit Dex-specific plaque formation of both IgM and IgA isotypes. Further indications for the sharing of idiotypic determinants between IgM and IgA isotypes were obtained in assays of Dex-immune sera, since a clear correlation between the amounts of idiotype-positive antibody and the amounts of the Dex-specific IgA antibody could be detected (to be published).

### Discussion

It might be argued that the differential isotype patterns observed in TD and TI responses are inherent properties of the distinct B cell subpopulations partic-

TABLE IV  
*Dex-specific PFC of the IgM and IgA Isotypes Express the Same Recurrent Antibody Idiotype*

	Inhibitor	Dex-specific PFC/spleen			
		IgM	%	IgA	%
Mouse 1	None	30,600		4,500	
	à17-9 1/36	8,660	72	2,880	36
Mouse 2	None	35,880		5,720	
	à17-9 1/36	22,246	38	2,803	51

C57BL/6 mice were immunized with 10 µg Dex i.v. and assayed for Dex-specific IgM and IgA PFC in the absence or in the presence of indicated final dilution of anti-17-9 idiotypic antiserum (See Materials and Methods). % denotes the percentage of PFC specifically inhibited by the antiidiotypic antiserum.



ipating in the responses. Alternatively, they may be due to accessory cell-dependent regulatory signals influencing B cell switches. Recent experiments indicate that resting, IgM-bearing B lymphocyte clonal precursors are multipotent as to the expression of the various non-IgM isotypes (35). Furthermore, although B cells activated by lipopolysaccharide (LPS) give rise to clonal progenies secreting IgM, IgG3, and IgG2b preferentially, while specific T helper cells activate switches to IgG1, IgG2a, and IgA secretion, further analysis has indicated that the B cells activated by either T cells and LPS, are equally efficient in producing all isotypes (18, 19, 42) excluding any precommitment in the switching ability of B cells. It would appear that isotype commitment is regulated by the quality of the stimuli inducing terminal maturation in proliferating B cells (43).

Thymus-independent polysaccharides of repetitive epitopes have long been considered to induce IgM antibodies exclusively (44). Only recently it was shown that polysaccharides also induce the production of large amounts of IgG3 antibody (13, 45), which is the least represented IgG class in normal sera (46) and "background" PFC (47). In addition to IgM, IgG3 and IgG2b antibody classes are currently considered the prototype responses to TI antigens (11-13). In contrast, TD antigens preferentially induce the production of IgG1 and IgA antibody (14-17). Other observations, such as the reduced levels of circulating IgA in nude mice (34), and the absence of mitogen-dependent IgA responses *in vitro* and *in vivo* (20, 48) support the strict T cell dependence of IgA production.

The participation of accessory cells in the responses to TI antigens has recently been demonstrated in various experimental systems. Mongini et al. (40, 41) have shown that T cells specifically enhance the levels of IgG2a and to a lower degree, of IgG2b anti-TNP antibodies in response to TNP-Ficoll. Similar results were reported by Kagnoff (36), who showed that T cells enhance the production of IgA antibody in the response to Dex B1355. Thus, T cells participating in the response to TI antigens can enhance the expression of "T cell-dependent" isotypes, as also shown by Golding and Rittenberg (21). At the present time, however, the specificities of such helper cells are not clear. Several can be proposed, such as for the carrier itself or for Ig determinants of the responding B cells, namely isotypic, allotypic, or idiotypic determinants.

In the present report, we have investigated the PFC isotypes in the response to Dex. Earlier studies in this system (22) had indicated large differences among "high-responder" mouse strains in the expression of IgG PFC in the absence of T cell-dependent regulation. These responses, therefore, appeared a model of choice to study genetic control of switches at the B cell level. Similarly to the response to the related antigen Dex B1355 (36), however, a considerable fraction of the PFC induced by Dex produced IgA antibody, which on the day of optimal PFC response was roughly half of the IgM response. Furthermore, nude mice produced very low or undetectable amounts of non-IgM PFC and circulating antibodies to Dex, demonstrating that the expression of all non-IgM isotypes requires the presence of functional T cells. Since both the IgM and IgA PFC responses are maximal on day 5 after immunization, it appears likely that the IgA PFC arise from Dex-activated B cells switched directly to IgA secretion. Similarly to IgM antibody responses (26), the expression of non-IgM isotypes in Dex responses is characterized by a marked individual variability. Thus, not all

mice produce detectable amounts of the various isotypes, neither in PFC nor in serum antibody responses. The fact that littermates, kept under the same conditions, show this high degree of variability, may be a result of differential steady states of reactivity in the immune system, as previously argued (26).

In order to find indications on the specificity of the T cells involved in the isotype pattern observed, we studied the responses to Dex both as an epitope on a different (TD) carrier, and as a TI carrier to a different epitope. Immunization with Dex linked to the carrier BSA led to increased levels of "T cell-dependent" isotypes, IgG1 and IgG2a. Since also in this response, the "T cell-independent" isotypes IgG3 and IgG2b are well represented and, as shown above, these are in fact T cell-dependent, it appears that two levels of T cell help are involved, both carrier-specific, but requiring different thresholds of helper cell stimulation. Interestingly, in both cases, a marked IgA response is detected, even under conditions of limited carrier-related T cell activity.

Most important, immunization with DNP-Dex did not lead to expression of the same isotypes as those induced by Dex itself. While the less helper-dependent isotypes are produced, no IgA response is observed. This could be due to abrogation of some properties of the carrier Dex upon conjugation with DNP. On the other hand, the same doses of DNP-Dex do induce DNP-specific PFC of IgA and all IgG isotypes in BALB/c mice, making this possibility very unlikely. Alternatively these results could indicate that the T cell activities induced by Dex are not competent to interact with DNP-specific B cells. These data, taken together, would then indicate that helper activities induced by Dex do discriminate between Dex- and DNP-specific B cells. Most strikingly, the IgA PFC response is epitope-specific and exclusively observed to Dex, even when DNP is coupled to a protein carrier. It would follow that immunization with Dex epitopes gives rise to helper activities that are specific to the anti-Dex B cells and that induce them to the production of IgA. In support of this conclusion, we might add that we have failed to demonstrate Dex-specific T cells in mice immunized with Dex in a number of different protocols.

We suggest, therefore, that some of the T cells participating in the isotype switch of Dex-specific B cells are idiotypic-specific. We have previously shown that T cells, specific for a recurrent idiotypic do exist "naturally" in C57BL/6 mice (32), and as shown here both IgM and IgA Dex-specific PFC, induced by the antigen itself, express the same dominant idiotypic. Since the presence of these idiotypic-specific T cells appears to be controlled by allotype- and MHC-linked genes (49), it will be interesting to investigate the genetic control of the IgA anti-Dex responses.

### Summary

The isotype distribution of Dextran B 512 (Dex)-specific plaque-forming cells (PFC) and serum antibodies was studied after *in vivo* immunization in C57BL/6 mice. Although IgG2b and IgG3 could also be detected in most individuals, the majority of non-IgM PFC were of the IgA isotype. All classes other than IgM were T cell-dependent, as shown by their complete absence in athymic "nude" mice. This unusual isotype pattern was further investigated by studying the antibody responses to the same Dex epitope coupled to a protein carrier, and to

a different hapten coupled to the carrier Dex or to a protein. The results show that IgA responses are epitope-related and selectively associated with anti-Dex antibodies: no IgA PFC are detected against a hapten coupled to Dex or proteins, while the enhanced levels of helper cell reactivity provided by protein carrier to Dex result in the appearance of IgG1 antibodies in addition to IgA. These results indicate that T cells that modulate isotype patterns in these responses can discriminate between Dex- and DNP-specific B cells in the response to the same carrier. Since the same idiotype is detected on a large fraction of the IgM and IgA anti-Dex response and antiidiotypic helper cells have previously been detected in normal C57BL/6 mice, we suggest that idiotype-specific T cells control the production of IgA antibodies upon immunization with Dex.

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