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# Unresponsiveness following immunization with the T-cell-independent antigen dextran B512. Can it be abrogated?

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## SUMMARY

The bacterial carbohydrate dextran B512 is a thymus-independent (TI) antigen and a poor immunogen. Humoral responses consist primarily of IgM and no memory response is observed; rather, secondary responses to native dextran are similar to or suppressed compared with primary responses. However, immune responses to dextran can be enhanced. In this study we have used a protein–dextran conjugate that elicits a thymus-dependent (TD) immune response against dextran. Furthermore, we used the potent adjuvant cholera toxin (CT) for the dextran immunizations. This enables us to re-evaluate the phenomenon of poor secondary response to dextran and whether it can be abrogated. We show that native dextran-primed mice were not able to mount IgG anti-dextran antibody responses after repeated immunizations with the TD, protein–dextran conjugate. This was also apparent in the spleen, where almost no dextran-specific germinal centres were detected. However, the anti-protein antibody response was normal in these mice, demonstrating that it is only the anti-dextran-responding cells that are affected. The effect of CT adjuvant on these events was also evaluated. CT enhanced the humoral IgM anti-dextran responses as well as the splenic responses to dextran. But, the isotype profile was not altered, still no IgG was produced. In contrast, mice primed with the TD conjugate and repeatedly re-immunized with native, TI, dextran generated IgG anti-dextran responses. Our results indicate that it is probable that the lack of proper costimulation in the initiation of the response to dextran causes the suppressed secondary dextran responses. Furthermore, these results suggest that TI and TD forms of dextran activate the same type of B cells, since TI dextran-priming abrogated TD dextran IgG responses. The importance of the priming event for the induction of a classical memory response to carbohydrate antigens and the implications for vaccination strategies, are discussed.

## INTRODUCTION

Antigens that are able to activate B cells to antibody production without the assistance of T cells are referred to as thymus-independent (TI) (reviewed in ref. 1). TI antigens represent a large heterogeneous group and can be further subclassified into TI-1 and TI-2 antigens. Responsiveness to TI-2 antigens appears late in ontogeny and is impaired in CBA/N mice that carry an X-linked immune B-cell defect (*xid*).[2] In general, humoral immune responses to TI-2 antigens are characterized by primarily IgM secretion, minor affinity maturation and no development of memory responses (reviewed in. ref. 3).

Many TI-2 antigens are carbohydrates. We are frequently exposed to carbohydrate antigens since many bacteria and viruses are surrounded by a carbohydrate capsule or a glycosylated envelope, respectively.[4] Unfortunately, immune responses to carbohydrates are generally poor. Carbohydrates have repeating antigenic epitopes, large molecular weight (MW) and are resistant to degradation *in vivo*, factors which make it difficult for the immune system to process and recognize these molecules.[5,6] Pure carbohydrates cannot bind to or be presented on major histocompatibility complex (MHC) molecules and are therefore unrecognized by conventional T cells.[7,8] This entails that specific immune responses to carbohydrates are restricted to the B-cell compartment. Thus, the carbohydrate-specific B cells will probably not receive the proper T-cell costimulation and consequently will not differentiate into memory B cells.

B-cell memory is presumed to develop in the specialized

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Abbreviations: CSA, chicken serum albumin; CT, cholera toxin; Dx, dextran; FDC, follicular dendritic cell; FITC, fluorescein isothiocyanate; GC, germinal centre; MW, molecular weight; PNA, peanut agglutinin; TD, thymus dependent; TI, thymus independent; TR, Texas red.

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microenvironment in the B-cell follicles of lymphoid tissues called germinal centres (GC).[9,10] Antigen-activated B cells, which have received the proper costimulus from antigen-specific T cells, migrate into the B-cell follicles where GC are developed. In the GC, the B cells undergo rapid clonal expansion and somatic hypermutation of their immunoglobulin variable genes.[11–13] B cells that express high-affinity receptors for the antigen are positively selected by binding to antigen trapped in immune complexes on follicular dendritic cells (FDC),[14] and receive further costimulation by antigen-specific $CD4^+$ T cells present in the GC. These B cells continue to differentiate into plasma cells or memory cells, while B cells with low affinity for the antigen die by apoptosis.

The GC reaction is thought to occur in responses to thymus-dependent (TD) protein antigens. However, we and others have demonstrated that some TI antigens actually induce GC formation as well.[9,15–17] Primary immunization with a TI antigen, the bacterial polysaccharide native dextran B512 induced GC formation to the same extent as a TD protein–dextran conjugate, which suggested that TI and TD antigens do not necessarily differ in their ability to induce GC development.[17]

In spite of its ability to induce GC development, dextran B512 is a poor immunogen. Anti-dextran antibody responses consist primarily of IgM and no memory response is observed. Secondary antibody responses to native dextran are similar to or suppressed compared to primary responses.[18–20] This could also be observed *in situ* in the spleen where there were almost no dextran-specific GC following secondary responses.[17] One of the explanations for the suppressed secondary immune responses could be exhaustive proliferation of the responding B-cell population, that could occur when B-cell activation by an immunogenic dose of polysaccharide antigen takes place in the absence of generation of memory cells.[21,22]

We and others have shown that it is possible to induce TD responses to dextran by conjugating small, non-immunogenic, dextran molecules to a protein carrier.[23,24] Also, we have demonstrated that in contrast to most conventional adjuvants, cholera toxin (CT) is an efficient adjuvant for native dextran.[17,25] CT enhanced the splenic response as well as the humoral response to both TI and TD forms of dextran. These two different approaches to enhance dextran immunogenicity enable us to re-evaluate the phenomenon of poor secondary responses to dextran and whether the unresponsiveness could be abrogated and memory responses improved.

In this study we show that native, TI, dextran-primed mice were not able to mount IgG anti-dextran antibody responses after repeated immunizations with the protein–dextran, TD, conjugate. This correlated with the splenic findings, where there were almost no dextran-specific GC detected in the sections. However, the anti-protein antibody response was normal in these mice, demonstrating that it is only the anti-dextran-responding cells that are silenced. The effect of CT adjuvant on these events was also evaluated. CT induced an enhanced splenic response and it was possible to detect many dextran-specific GC in sections from mice that were primed with TI dextran and re-immunized with TD dextran. Anti-dextran IgM levels were increased in these mice but the coadministration of CT did not alter the isotype profile of the response, still no IgG was produced. In contrast, mice primed with the TD conjugate and repeatedly re-immunized with native, TI, dextran generated IgG anti-dextran responses. The differences in priming with a TI and a TD antigen are discussed.

## MATERIALS AND METHODS

### *Animals*

C57BL/6 mice were bred under full-barrier conditions at Charles River (Uppsala, Sweden). The mice were maintained in our animal facilities at Stockholm University and were 2–4 months old when used in the experiments.

### *Antigens and adjuvants*

As a TI-2 antigen, we used native dextran B512 with a MW of $5 \times 10^6$–$40 \times 10^6$ (INC Pharmaceuticals Inc., Cleveland, OH). To obtain the TD protein–dextran conjugate, dextran with a MW of $10^3$ (3–5 glucose units) was conjugated to the protein chicken serum albumin (CSA) (Sigma Chemical Co., St. Louis, MO). Dextran was conjugated to hydrazide-modified CSA via its terminal aldehyde group using reductive amination[23] and was kindly provided by Christian Krog-Jensen, Department of Organic Chemistry, Stockholm University. CT was obtained from List Biological Laboratories Inc. (Campbell, CA).

### *Immunizations*

Mice were immunized intraperitoneally (i.p.) with the different antigens in different combinations. Native dextran, 10 μg/mouse, was administered in saline with or without CT. CSA–Dx, 100 μg/mouse, was administered precipitated in alum or soluble in saline with CT. CT was administered i.p. together with the different antigens, each mouse receiving 1 μg CT per dose. For studies of secondary and tertiary responses, animals were immunized 28 days after primary immunization and 35 days after secondary immunization, respectively. Mice were bled by retro-orbital puncture under light ether anaesthesia 10 and 28 days after primary immunization and 7 days after secondary or 7 days after tertiary immunizations and spleens were removed after bleeding, both after secondary and tertiary immunizations. Serum was separated after centrifugation and tested in enzyme-linked immunosorbent assay (ELISA).

### *Detection of anti-dextran and anti-CSA antibodies in serum with ELISA*

The ELISA were performed as described.[26] Briefly, ELISA plates (Costar, Cambridge, MA) were coated with 10 μg/ml dextran T250 (Pharmacia Fine Chemicals) or 10 μg/ml CSA (Sigma). Test sera were added in twofold dilutions, starting with 1/100 dilution. Dextran-specific mouse monoclonal antibodies with known concentrations were used as positive controls for the dextran ELISA. Bound immunoglobulin was detected with alkaline phosphatase-labelled goat anti-mouse IgM, IgG, IgG1 and IgG3 (Southern Biotech. Assoc., Birmingham, AL) and *p*-nitro-phenyl phosphatase substrate (Sigma). Optical density (OD) values at 405 nm were determined using an Anthos Reader 2001 (Anthos Labtech Instruments, Salzburg, Austria).

### *Preparation of splenic sections and* in situ *immunofluorescence*

After removal, spleens were immediately frozen in liquid nitrogen and stored at −70°. Spleens were embedded in Tissue

Tek OCT compound (Miles Inc. Elkhart, IN) and cryostat sections (6 μm) were cut and mounted. The slides were air-dried for 30–60 min and stored at −70° until use. Cryostat sections were fixed for 15 min in ice-cold acetone. Subsequently slides were rinsed with phosphate-buffered saline (PBS) and blocked with horse serum (5% in PBS) for 30 min. Sections were stained with fluorescein isothiocyanate (FITC)-conjugated dextran (FITC–Dx) 250 000 MW (purchased from Pharmacia Fine Chemicals or Sigma) and biotin-conjugated peanut agglutinin (bi-PNA) developed with streptavidin–Texas Red (TR) conjugate (Vector Laboratories Inc. Burlingame, CA). This double-staining has been shown specifically to detect dextran-binding GC B cells, by analysis of PNA/FITC–Dx double-stained spleen cells from dextran-immunized mice with multiparameter fluorescence-activated cell sorter (FACS).[15] The sections were incubated with biotinylated reagents as indicated for 60 min and then incubated with fluoresceinated reagents and avidin-conjugated TR for 60 min with washings after each staining. All stainings were performed in a humidified chamber protected from light.

## RESULTS

### Priming with native (TI) dextran abolishes future IgG anti-dextran antibody production, even after repeated immunizations with a TD protein–dextran conjugate

C57BL/6 mice were immunized once with native dextran and boosted once or twice with the optimal TD protein–dextran conjugate CSA–Dx precipitated in alum adjuvant. Serum was collected and mice were killed 7 days after secondary or tertiary immunizations. Anti-dextran and anti-CSA antibodies were detected in antigen-specific ELISA. We have previously demonstrated that this is a suitable time-point to detect optimal anti-dextran antibody levels, independently of the immunization form.[17] To identify dextran-specific GC B cells, splenic sections were prepared as described in the Materials and Methods followed by staining of the sections with bi-PNA (developed with avidin–TR) and FITC–Dx, as described by Wang *et al.*[15]

A single immunization with native dextran elicits a modest but clearly detectable GC response in the spleen. Many areas with dextran-binding cells can be detected both in and outside the GC (Table 1). Mice that were primed with native dextran and later immunized with CSA–Dx had very few GC in their spleens and there were only a few areas of dextran-binding cells in the sections, which is similar to what is observed after repeated native dextran immunizations (Table 1). A secondary immunization of the native dextran primed animals with CSA–Dx enhanced the splenic response but the majority of GC were not specific for dextran. Most probably, these GC were specific for the protein-part of the CSA–Dx conjugate (Table 1). Serum samples from these mice were tested for anti-dextran antibodies in a dextran-specific ELISA. Although IgM levels increased after CSA–Dx immunization (Fig. 1a), IgG anti-dextran antibody titres were below detection in all samples, also in the mice that received repeated CSA–Dx boosters (Fig. 1b). This is in sharp contrast to mice that have received CSA–Dx twice without a primary immunization with native dextran, which readily produce IgG anti-dextran antibodies (Fig. 1b).

**Table 1.** Summary of the splenic response after primary immunization with native dextran and secondary and tertiary immunizations with CSA–Dx, in the absence or presence of CT

| Immunization* | CT | GC† | $Dx^{+}$‡ |
|---|---|---|---|
| *Absence of CT* | | | |
| 1° native dextran | – | 3·7±0·7 | 7·5±2·0 |
| 1° native dextran/2° native dextran | – | 1·0±0·6 | 1·9±0·7 |
| 1° native dextran/2°CSA–Dx | – | 1·4±0·7 | 3·2±0·5 |
| 1° native dextran/2°CSA–Dx/ 3°CSA–Dx | – | 4·2±1·6 | 1·0±0·5 |
| *Presence of CT* | | | |
| 1° native dextran/2°CSA–Dx | + | 10·3±0·6 | 5·9±1·2 |
| 1° native dextran/2° native dextran | + | 8·2±2·0 | 8·5±3·0 |
| 1°CSA–Dx/2°CSA–Dx | + | 8·2±0·7 | 3·5±0·7 |

*Mice were immunized as described in the Materials and Methods and killed 10 days after primary or 7 days after secondary or tertiary immunization.

†Number of PNA-positive areas per section. Values represent a mean of results from two mice in one out of two similar experiments.

‡Number of areas stained with FITC–Dx in the sections. Values represent a mean of results from two mice in one out of two similar experiments.

The anti-CSA antibody response however, was perfectly normal with anti-CSA IgG levels that increased after repeated CSA–Dx immunizations, showing that it was only the anti-dextran response that was affected by the priming with native dextran (Fig. 2).

### CT adjuvant enhances the immune response, but does not alter the isotype profile of the antibody response induced by priming with native (TI) dextran

We have previously demonstrated that CT is an efficient adjuvant for immune responses to dextran, both in terms of enhancing the splenic response and the humoral response. We wanted to find out if CT could provide some stimulatory signals, possibly during the priming event, that could induce IgG production after CSA–Dx immunization in native dextran-primed animals. Mice were primed with native dextran and re-immunized with CSA–Dx. As controls, mice receiving two immunizations with native dextran or CSA–Dx were included. CT was administered together with the antigens, both in the primary and the secondary immunizations. There was an enhanced GC response in the spleens from the mice that were immunized in the presence of CT (compare sections of Table 1). This enhancement is not due to a a CT-promoted prolongation of the primary response, because late primary GC responses were declining to a similar extent in the presence or absence of CT.[17] IgM and IgG levels after immunizations in the presence of CT are shown in Fig. 3. IgM was enhanced in all groups compared to the mice that did not receive CT (compare Figs 1a and 3a). However, it was not possible to detect IgG production in native dextran-primed mice (Fig. 3b). This shows that although the GC response is enhanced and IgM anti-dextran antibody levels are increased, CT cannot alter the arrest at the IgG level induced by native dextran priming.

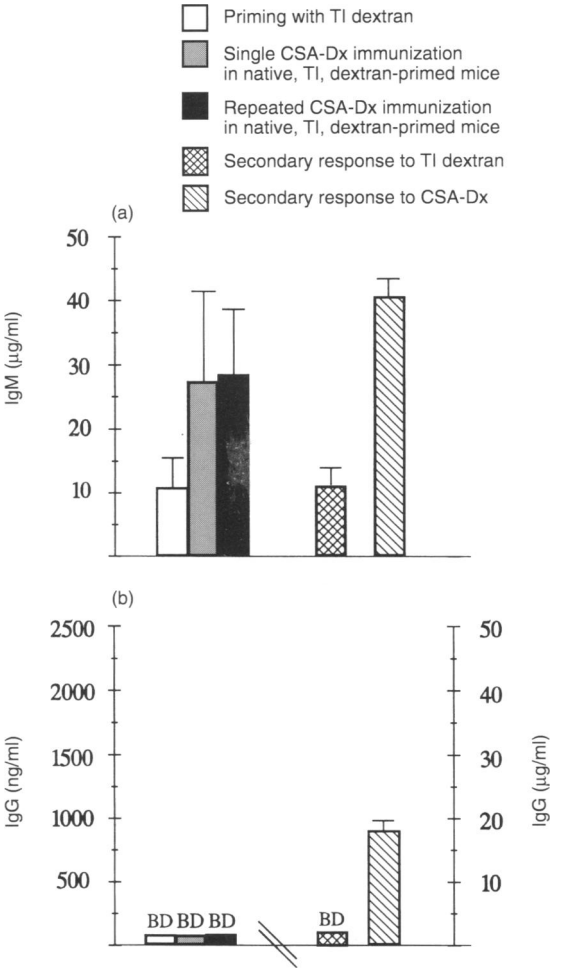


**Figure 1.** Priming with native (TI) dextran abolishes future IgG anti-dextran antibody production, even after repeated immunizations with a TD protein–dextran conjugate. C57BL/6 mice were primed with 10 μg/ml native dextran in saline and later immunized twice with 100 μg CSA–Dx in the presence of alum adjuvant. The mice were bled 10 days after primary immunization and 7 days after secondary immunization and serum samples were tested in ELISA. Data are shown as μg/ml anti-dextran IgM (a) and ng/ml or μg/ml anti-dextran IgG (b) antibodies after priming with native dextran, after a single immunization with CSA–Dx in native dextran-primed mice and repeated CSA–Dx immunizations in native dextran-primed mice. As controls, secondary IgM and IgG responses to native dextran and CSA–Dx are shown. Note the different scales on the *y*-axes in (b). BD, below detection; data shown represent mean values of two to four mice/group, with error bars indicating standard deviations.

### Priming with a TD form of dextran results in optimal TD anti-dextran responses even after repeated boosters with a TI form of dextran

The reverse experiment was performed, and the mice were primed with the optimal TD protein–dextran conjugate followed by one or two native dextran boosters. The splenic sections from these mice contained many GC which to a large extent were dextran-specific (Table 2). Interestingly, anti-dextran IgG antibodies were produced, also in the mice that were boosted twice with native dextran (Fig. 4). This demonstrates the importance of the priming event in an immune response since mice that are immunized twice with native dextran alone, without a previous TD, CSA–Dx, priming have a suppressed secondary anti-dextran response with no dextran-binding B cells present in the spleen and almost no IgG anti-dextran production.[17,25]

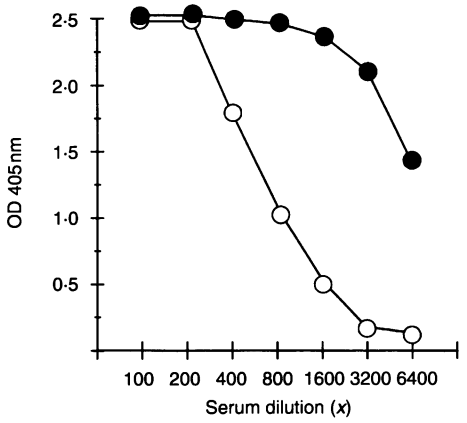


**Figure 2.** The antibody response to CSA is normal in native dextran-primed mice; see legend to Fig. 1. Primary IgG anti-CSA antibody response after a single CSA–Dx immunization in native dextran-primed mice (○) and secondary IgG anti-CSA antibody response after two CSA–Dx immunizations in native dextran-primed mice are shown (●).

**Table 2.** Summary of the splenic response after primary immunization with CSA–Dx and secondary or tertiary immunizations with native dextran

| Immunization* | GC† | Dx⁺‡ |
|---|---|---|
| 1° CSA–Dx | 4·3±0·5 | 1·3±0·4 |
| 1° CSA–Dx/2° CSA–Dx | 5·0±0·9 | 1·8±0·8 |
| 1° CSA–Dx/2° native dextran | 5·8±1·8 | 4·9±0·1 |
| 1° CSA–Dx/2° native dextran/ 3° native dextran | 4·2±0·7 | 7·5±3·5 |

*Mice were immunized as described in the Materials and Methods and killed 10 days after primary and 7 days after secondary or tertiary immunization.

†Number of PNA-positive areas per section. Values represent a mean of results from two mice in one out of two similar experiments.

‡Number of areas stained with FITC–Dx in the sections. Values represent a mean of results from two mice in one out of two similar experiments.

## DISCUSSION

Initial steps of antigen-specific activation of B cells in the lymphoid organs include binding of native antigen and costimulation provided by T cells. Once activated, the B cells either differentiate into antibody-producing plasma cells or migrate into the primary lymphoid follicles and undergo affinity maturation in the GC. A major goal for the GC reaction is to provide the organism with B cells that have higher affinity for the antigen that initiated the reaction and to provide memory cells for future exposure of the same antigen. Interaction with antigen retained in immune-complexes on follicular dendritic cells selects B cells with high affinity for the antigen. Antigen-specific T cells present in the

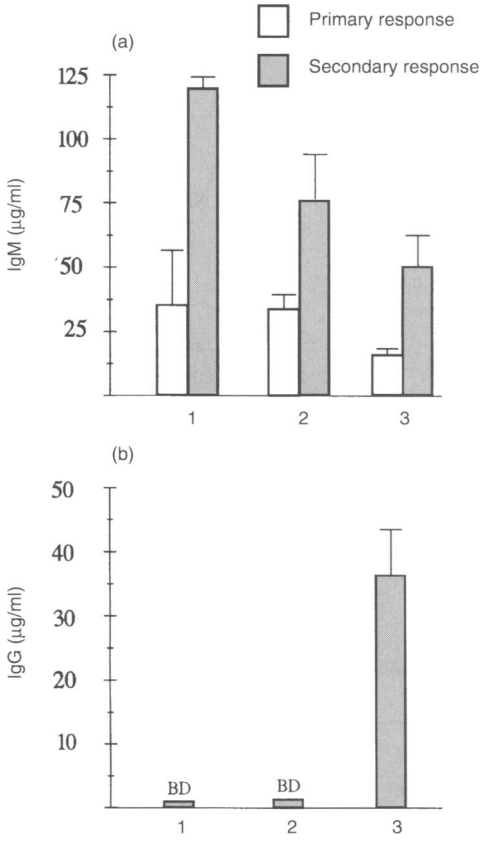


**Figure 3.** CT adjuvant does not alter the isotype profile of the antibody response induced by priming with native (TI) dextran. C57BL/6 mice were primed with 10 μg/ml native dextran administered together with CT adjuvant and later immunized twice with 100 μg CSA–Dx in the presence of CT adjuvant. The mice were bled 10 days after primary immunization and 7 days after secondary immunization and serum samples were tested in ELISA. Data are shown as μg/ml anti-dextran IgM (a) and IgG (b) antibodies after priming with native dextran followed by a secondary immunization with CSA–Dx (group 1), after two immunizations with native dextran (group 2) and after two CSA–Dx immunizations (group 3). BD, below detection; data shown represent mean values of two mice/group in one out of two similar experiments, with error bars indicating standard deviations.

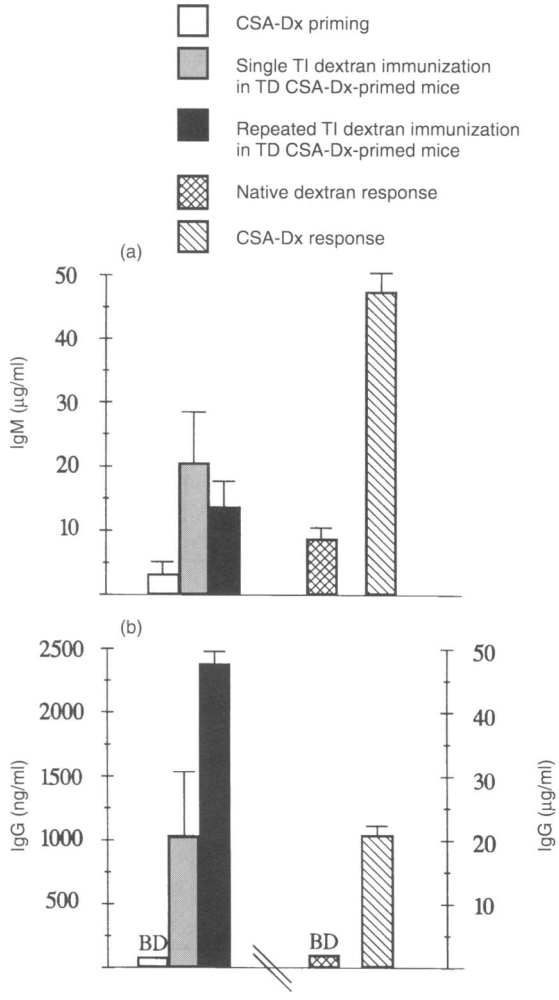


**Figure 4.** Priming with a TD form of dextran results in optimal TD anti-dextran responses even after repeated boosters with a TI form of dextran. C57BL/6 mice were primed with 100 μg/ml CSA–Dx in alum adjuvant and later immunized twice with 10 μg native dextran in saline. The mice were bled 10 days after primary immunization and 7 days after secondary immunization and serum samples were tested in ELISA. Data are shown as μg/ml anti-dextran IgM (a) and ng/ml or μg/ml anti-dextran IgG (b) antibodies after priming with CSA–Dx, after a single immunization with native dextran in CSA–Dx-primed mice and repeated native dextran immunizations in CSA–Dx-primed mice. As controls, secondary IgM and IgG responses to native dextran and CSA–Dx are shown. Note the different scales on the *y*-axes in (b). BD, below detection; data represent mean values of two to six mice/group, with error bars indicating standard deviations.

GC then provide further costimulation to the selected B cells, which differentiate to either plasma cells or memory B cells.

The GC reaction has mainly been described for TD antigens. However, we and others have demonstrated that some TI antigens, such as native dextran, could induce GC formation to the same extent as a TD form of the same antigen. However, the outcome of these GC reactions was markedly different. The secondary antibody response to the TD form of dextran was vigorous with high IgG anti-dextran antibody production, while the secondary splenic and humoral responses to the TI form, native dextran, showed no signs of maturation.[24] Actually the secondary response to native dextran could be described as suppressed compared to the primary response, a phenomenon that could be explained by exhaustive proliferation (clonal deletion) of the responding B cells. This could occur when B-cell activation by an immunogenic dose of carbohydrate antigen takes place in the absence of generation of memory cells.[21,22] No memory is generated due to the T-cell ignorance of the carbohydrate.

In this study we wanted to re-evaluate the absence of memory responses to dextran B512. This was possible by using two different potent inducers of anti-dextran responses. The protein–dextran conjugate CSA–Dx is able to induce an efficient TD antibody response to dextran. The exposure of the dextran molecules in this conjugate is reminiscent of the organization of carbohydrates in the whole bacteria where terminal non-reducing ends are exposed. This could possibly facilitate recognition and processing of the molecule thereby making it more immunogenic. Consequently, CSA–Dx evokes

a good secondary anti-dextran antibody response. To have a possibility directly to affect the response to native dextran we included CT as an adjuvant for the immunizations. We have previously demonstrated that coadministration of CT for native dextran immunizations, enhances both secondary splenic and humoral IgM responses to dextran.

Mice that were primed with native, TI, dextran did not produce IgG anti-dextran antibodies after repeated immunizations with the TD CSA–Dx conjugate and the splenic responses were low. The anti-CSA antibody response was normal, demonstrating that it was only the dextran-responding B-cell population that was affected by the TI dextran priming. Including CT as an adjuvant for the immunizations enhanced splenic GC responses and increased IgM anti-dextran antibody production. However, no IgG against dextran was produced. This correlated with our previous findings with repeated native dextran challenge in the presence of CT.[17,25] It is possible that CT provides specific B cells with enough stimuli to remain in the body for future dextran challenge. It could be speculated that the B cells that are responsible for this enhanced secondary IgM production, are memory B cells. They would differ from conventional memory cells in that they do not switch to IgG, which could be due to different activation signals delivered to the B cells. This implies that signals for maturation and for immunoglobulin class switch induction are of different kinds. This is in agreement with other reports describing IgM memory TI-2 antigen responses induced by using stimulating molecules like lipopolysaccharide as adjuvant.[27–29] In addition, it has been reported that some TI-2 antigens that are capable of GC induction have been reported to evoke non-classical T-cell help.[16] It has been shown that some T cells of the $\gamma/\delta$ lineage can recognize phosphate groups in association with polysaccharide epitopes.[30] The CT-induced up-regulation of the costimulatory molecules CD80 and/or CD86 could also explain the enhanced response.[31]

This is of interest to consider in relation to the recent finding of a subset of human tonsillar $IgD^+$ B cells which has the phenotypical and functional characteristics of GC B cells but does not undergo isotype switching.[32] The authors speculated that this subset could be representative of a GC population generated in response to bacterial carbohydrate antigens. It could be argued that the B cells responding to native dextran are of a different kind than the B cells responding to the TD protein–dextran conjugate. It has been shown that another type of dextran, B1355S ($\alpha$1-3, $\alpha$1-6), is able to activate B cells of the $CD5^+$ lineage.[33] Previous experiments performed in our laboratory with high, tolerizing (1 mg/dose) doses of native dextran also indicated that TI and TD dextrans activated distinct B-cell populations.[34,35] However, the results obtained in this study, where we have studied the behaviour of cells activated by an immunogenic dose of dextran indicate that the same B-cell population is able to respond to both TD and TI forms of the same antigen, as was previously suggested,[36] although we cannot formally exclude that native dextran B512 does not activate a separate B-cell subset. Immunizations with CSA–Dx did not induce a TD anti-dextran antibody response in native, TI, dextran-primed animals. Interestingly, in mice that were primed with a single dose of CSA–Dx, we observed IgG anti-dextran responses even after two immunizations with native dextran. Also, many dextran-specific GC were observed in the spleens. This suggests that TI and TD dextran activate the same B-cell population, and also demonstrates the importance of an efficient priming (TD) for future carbohydrate (TI) challenges.

There exist other types of regulation of immune responses to dextran as well. Primary anti-dextran responses are followed by the induction of anti-idiotypic antibodies that are able to, at least in part, abolish a response after repeated immunization.[37]

A better understanding of the priming event could have implications for vaccination against glycosylated microorganisms. Conjugating carbohydrates to suitable protein carriers and/or use of costimulatory agents could make the recognition and the response to glycopeptides and carbohydrates more efficient. We show, that if the immune response is properly primed with a TD form of the carbohydrate, subsequent challenges with the same carbohydrate elicit adequate memory responses. Vaccines containing constructed synthetic peptides can induce antibodies that may be unable to recognize the original glycoprotein. It is therefore of great importance to find a way to make the immune system recognize and respond to these carbohydrates and/or glycopeptides. Polysaccharide vaccines have proved to be successful, but only in adult individuals. Immune responses to carbohydrates mature late in life and it is therefore important to find a way to vaccinate and protect children from infections caused by, for example, encapsulated bacteria.

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