Germinal center formation following immunization with the polysaccharide dextran B512 is substantially increased by cholera toxin

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Abstract

We have compared the splenic responses following immunization with the T cell-independent (TI)-2 antigen native dextran B512 and with a thymus-dependent (TD) protein–dextran conjugate. Interestingly, primary immunization with native dextran induced germinal center (GC) formation in the spleen to the same extent as the protein–dextran conjugate. The GC were antigen-specific as characterized by the presence of peanut agglutinin (PNA)-positive areas that were also binding FITC-conjugated dextran. Dextran-binding B cells were also detected outside the GC as sites of antibody-producing cells. The secondary splenic response to native dextran was suppressed compared to the primary response, with almost no dextran-specific GC or extra-follicular sites with dextran-specific B cells present in the sections. This suppression could be reverted by using cholera toxin (CT) as an adjuvant for the dextran immunizations. Following native dextran immunization with CT adjuvant a secondary splenic GC response similar to a TD secondary splenic GC response was generated, with almost all the dextran-specific B cells located in the GC. Collectively, this indicates that the difference between TI and TD antigen responses is not due to different abilities in inducing GC development, rather the GC reaction is less productive for a TI antigen than for a TD antigen. CT can both increase secondary GC formation in particular for the TI form of dextran and ameliorate the GC reaction, as reflected by increased anti-dextran antibody levels.

Introduction

Antigenic stimulation induces the formation of germinal centers (GC) in lymphoid tissue. GC arise during B cell responses against T cell-dependent (TD) antigens and originate from rapid clonal expansion of a few founder cells. The importance of GC is demonstrated by their association with clonal selection of B cells, expansion of memory B cells, somatic hypermutation and differentiation to memory B cells or plasma cells (reviewed in 1,2). Some T cell-independent (TI) antigens can also induce GC formation (3–5). In many aspects, the cellular responses are similar for TI and TD antigens. In both cases there is an early accumulation of activated proliferating B cells in the periartereolar lymphoid sheath (PALS), and it has been demonstrated that TI and TD antigens induce GC reactions with very similar kinetics during the primary response (6).

TI antigens were originally defined by their capacity to activate B cells without the involvement of T cells and have been further subdivided into type 1 (TI-1) and type 2 (TI-2) antigens depending on their ability to evoke a response in the immunodeficient CBA/N mouse strain. The capsular carbohydrates of pathologically important bacteria such as Streptococcus pneumoniae and Haemophilus influenzae b are...
TI-2 antigens. Carbohydrates frequently generate restricted immune responses characterized by dominant expression of a few V\textsubscript{H} and/or V\textsubscript{L} genes. In terms of antibody responses, primarily IgM is produced and there is no development of immunological memory (reviewed in 7). Infants under 2 years, the elderly and immunosuppressed individuals generate poor immune responses against these carbohydrates, and are therefore very sensitive to bacterial infections caused by encapsulated bacteria (8).

In order to study the complex immune responses against carbohydrates we are using native dextran B512. It is extracted from the bacteria *Leuconostoc mesenteroides* and is a highly repetitive molecule consisting of glucose units linked in α(1–6) position. It has an average mol. wt of 5×10\textsuperscript{7} and is a TI-2 antigen in this native, high mol. wt form, but the immunogenicity of dextran decreases with decreasing mol. wt (9–12). However, it is possible to obtain a TD immune response against dextran, with a good memory response and high anti-dextran IgG titers, by conjugating non-immunogenic carrier (13–14).

We have recently shown that it is possible to enhance the antibody response against dextran by using cholera toxin (CT) as an adjuvant (15). Antibody responses against TI as well as TD forms of dextran were enhanced. The anti-dextran antibody responses were significantly improved with CT adjuvant, particularly the secondary response. The precise mechanisms for its adjuvant effects in vivo are still unknown but CT is a very potent adjuvant for both mucosal and systemic responses for many different antigens (16,17). It has been demonstrated that CT augments antigen presentation (18) and increases the frequency of antigen-specific T cells (19). Further, CT has direct effects on B cells showed by promotion of isotype differentiation of purified B cells and in a clonal B cell population (20–21).

In this study we have continued to investigate the effects of CT on the immune response to dextran at the site of the immune reaction, in order to investigate whether the increased antibody response was correlated to histological changes in the spleen. We compared GC formation, presence of dextran-specific B cells within as well as outside GC and deposition of dextran in the sections following both primary and secondary immunizations with TI and TD forms of dextran. The effect of CT adjuvant on these events was evaluated. Primary immunization with native dextran induced GC formation in the spleen to the same extent as the protein–dextran conjugate. The GC were dextran specific as visualized by double staining with biotin-conjugated PNA and FITC-conjugated dextran, but dextran-specific B cells could also be detected outside the GC. The secondary GC response to native, TI, dextran was suppressed compared to the primary response and almost no GC or extra-follicular sites with dextran-specific B cells were detected in the splenic sections. In sharp contrast to this, when CT was used as an adjuvant for the immunizations with native dextran this suppression was abrogated and a GC response similar to a TD secondary splenic GC response was observed. This was characterized by a high number of dextran-specific GC and almost all dextran binding cells in the spleen were located in the GC. Consequently, CT can both enhance secondary GC formation in particular for the TI form of dextran and ameliorate the GC reaction, as reflected by increased anti-dextran antibody levels.

**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 immunizations**

Native dextran B512 (TI-antigen) with a mol. wt of 5–40×10\textsuperscript{6} was obtained from INC Pharmaceuticals (Cleveland, OH). A TD form of dextran was obtained by conjugating dextran with a mol. wt of 10\textsuperscript{5} (3–5 glucose units) to the protein chicken serum albumin (CSA) (Sigma, St Louis, MO). Dextran was conjugated to hydrazide-CSA via its terminal aldehyde group using reductive amination and was kindly provided by Dr Christian Krog-Jensen (Department of Organic Chemistry, Stockholm University). CT was obtained from List Biological Laboratories (Campbell, CA). Mice were immunized i.p. with 10 µg of native dextran or with 100 µg of CSA–Dx. Both antigens were administered in soluble form, precipitated in alum adjuvant or together with CT. CT was administered i.p. together with the different antigens, each mouse receiving 1 µg CT per dose both for primary and secondary immunizations.

**Detection of anti-dextran antibodies in serum with ELISA**

The ELISA were performed as described (22). Briefly, ELISA plates (Costar, Cambridge, MA) were coated with 10 µg/ml dextran T250 (Pharmacia Fine Chemicals, Uppsala, Sweden). Test sera were added in 2-fold dilutions, starting with 1/100 dilution. Dextran-specific mouse mAb were used as positive controls. Bound Ig was detected with alkaline phosphatase-labeled goat anti-mouse IgM and IgG (Southern Biotechnology Associates, Birmingham, AL) and η-nitro-phenyl phosphatase substrate (Sigma). OD values at 405 nm were determined using an Anthos Reader 2001 (Anthos Labtech Instruments, Salzburg, Austria).

**Preparation of spleen sections and in situ immunofluorescence**

Spleens were removed after primary and secondary immunizations, and immediately frozen in liquid nitrogen and stored at −70°C. Different time points after immunization were tested based on the results from the humoral response. Day 10 after primary and day 7 after secondary immunization...
were within the peak of GC responses for all groups and are shown. Spleens were embedded in Tissue Tek OCT compound (Miles, Naperville, IL) and cryostat sections (6 µm) were cut and mounted. The slides were air-dried for 30–60 min and stored at −70°C until use. Cryostat sections were fixed for 15 min in ice-cold acetone. Subsequently slides were rinsed with PBS and blocked with horse serum (5%) in PBS for 30 min. Sections were stained with FITC-conjugated peanut agglutinin (PNA) developed with streptavidin–Texas Red and FITC–Dx (3).

All stainings were performed in a humidified method already used by Wang et al. where sections were co-stained with biotin–PNA (developed with avidin–Texas Red) and FITC–Dx (3).

**Results**

**GC formation in primary responses to dextran**

In order to compare GC formation following TI and TD dextran challenge we immunized C57BL/6 mice with native dextran (soluble) or the protein–dextran conjugate CSA–Dx (in alum). An initial kinetic analysis, where several time points were checked, was performed to find a time point where both splenic and humoral responses were optimized for all groups (not shown). For illustration of primary responses, the mice were sacrificed 10 days after primary immunization and splenic sections were prepared as described in Methods. To identify dextran-specific GC B cells, we used the staining method already used by Wang et al. where sections were co-stained with biotin–PNA (developed with avidin–Texas Red) and FITC–Dx (3).

Immunization with native dextran readily induced development of dextran-specific GC (PNA/Dx–FITC double-positive areas). Apart from the presence of dextran-specific B cells within the GC there were also many extra-follicular sites with dextran-specific B cells which probably constitute foci of antibody-producing cells (Table 1a, day 10). This is in agreement with the results obtained by Wang et al. (3). Immunization of the TD conjugate CSA–Dx induced GC formation to the same extent as native dextran. On the other hand, only a minority of the GC contained dextran-specific cells (Table 1a). This is to be expected since many of the GC formed after CSA–Dx immunization should be specific for CSA. Splenothers from unimmunized mice had almost no GC and were completely negative for FITC–Dx staining (Table 1a).

**Table 1. Summary of the primary and secondary splenic response to immunization with TI, native dextran without (a) or in the presence (b) of CT adjuvant**

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Kinetics</th>
<th>Day</th>
<th>( r^2 )</th>
<th>Dx*%</th>
<th>Dx*GCd</th>
<th>Percent in GC</th>
</tr>
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<tbody>
<tr>
<td>Non-immunized (a) Without CT</td>
<td>native DX</td>
<td>primary</td>
<td>10</td>
<td>3</td>
<td>7.5 ± 3.0</td>
<td>3.3 ± 1.6</td>
</tr>
<tr>
<td>native DX</td>
<td>primary</td>
<td>28</td>
<td>3</td>
<td>5.5 ± 1.3</td>
<td>2.0 ± 1.5</td>
<td>36</td>
</tr>
<tr>
<td>native DX</td>
<td>secondary</td>
<td>7</td>
<td>6</td>
<td>1.3 ± 1.0</td>
<td>0.8 ± 1.0</td>
<td>62</td>
</tr>
<tr>
<td>native DX in alum</td>
<td>secondary</td>
<td>7</td>
<td>2</td>
<td>7.0 ± 2.5</td>
<td>3.8 ± 2.0</td>
<td>54</td>
</tr>
<tr>
<td>(b) With CT</td>
<td>native Dx+CT</td>
<td>primary</td>
<td>10</td>
<td>3</td>
<td>10.3 ± 4.0</td>
<td>7.0 ± 1.7</td>
</tr>
<tr>
<td>native Dx+CT</td>
<td>primary</td>
<td>28</td>
<td>3</td>
<td>4.7 ± 1.3</td>
<td>2.9 ± 0.9</td>
<td>70</td>
</tr>
<tr>
<td>native Dx+CT</td>
<td>secondary</td>
<td>7</td>
<td>7</td>
<td>9.1 ± 3.3</td>
<td>8.3 ± 3.2</td>
<td>91</td>
</tr>
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</table>

- aMice were immunized as described in Methods and killed 10 or 28 days after primary immunization or 7 days after secondary immunization.
- bNumber of animals. Experiments were repeated 1–3 times with two to three mice per group per experiment.
- cNumber of areas stained with FITC–Dx in the sections. Three to six sections per mouse were evaluated. The mean results from each mouse were added together and averaged. SD values represent the variation in the means of the different mice used.
- dNumber of GC per section containing dextran-specific B cells. Three to six sections per mouse were evaluated. The mean results from each mouse were added together and averaged. SD values represent the variation in the means of the different mice used.
- ePercent of the total number of Dx–FITC binding B cell areas that were detected within the GCs.

**Table 2. Summary of the primary and secondary splenic response to immunization with the TD, CSA-Dx conjugate without (a) or in the presence (b) of CT adjuvant**

<table>
<thead>
<tr>
<th>Immunization( ^a )</th>
<th>kinetics</th>
<th>( r^2 )</th>
<th>Percent Dx*GC( ^d )</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Without CT</td>
<td>CSA–Dx in alum</td>
<td>primary</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>CSA–Dx in alum</td>
<td>secondary</td>
<td>2</td>
</tr>
<tr>
<td>(b) With CT</td>
<td>CSA–Dx+CT</td>
<td>primary</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>CSA–Dx+CT</td>
<td>secondary</td>
<td>2</td>
</tr>
</tbody>
</table>

- aMice were immunized as described in Methods and killed 10 or 28 days after primary or 7 days after secondary immunization.
- bNumber of animals.
- cThe data show one representative out of three similar experiments and are expressed as percent of the total number of GC that were specific for dextran.
Cholera toxin enhances TI germinal center formation
GC formation in secondary responses to dextran

The secondary antibody response to native dextran is similar or suppressed compared to the primary response (23,24) even if dextran is administered together with an adjuvant. To investigate if this suppression of the antibody response correlated with a diminished splenic response we stained splenic sections from mice immunized twice with native dextran in saline or precipitated in alum. We could observe a clear difference in the splenic responses depending on the immunization form. In mice immunized twice with soluble dextran there were few GC and there was an almost complete absence of dextran-specific B cells not only within the GC but also in the extra-follicular sites (Fig. 1A and B, and Table 1a). In sections from mice immunized twice with native dextran in alum many areas with dextran-specific B cells were detected. There were few GC and the dextran-specific B cells were preferentially located outside the GC (Table 1a). However, the secondary antibody response in these mice was not enhanced compared to the primary response (not shown).

The secondary GC response to CSA–Dx was slightly enhanced compared to the primary response. Although the secondary antibody response against CSA–Dx is characterized by high anti-dextran IgG titers (14) we could only observe few GC containing dextran-specific cells in the sections from secondary immunizations (Table 2a).

Effect of CT on the GC formation following immunizations with dextran

We have shown that the antibody response to both TI and TD forms of dextran could be enhanced by using CT as an adjuvant (Fig. 2) (15). To investigate if this modulation of the antibody response could be correlated with changes in the splenic response we examined the spleens of C57BL/6 mice immunized with our dextran preparations in the presence of CT.

The primary splenic responses to both antigens were marginally enhanced by CT (Table 1b and 2b) although the structure of the GC was more clearly defined and stained more intensely with PNA than those from mice that did not receive CT adjuvant.

The effect of CT was more prominent in secondary responses. After secondary immunization with the TI form of dextran, there was a marked increase in the number of dextran-specific GC compared to the primary response. Also in contrast to the primary response, there were very few areas of dextran-specific B cells outside the GC. Instead, almost 100% of the dextran-specific B cells in the secondary response were located within the GC (Fig. 1C and D, and Table 1b). This should be compared with the results obtained with spleens from mice that were immunized twice with dextran without CT, where there was almost a complete absence of dextran-specific GC in the sections. The enhanced secondary response was not due to an ability of CT to prolong the primary response. Late primary responses, measured at the day of secondary immunization, showed that the splenic response to dextran was diminishing in both groups (Table 1).

Secondary immunizations with CSA–Dx in the presence of CT induced formation of more and larger GC that stained more brightly than the ones seen in secondary responses to CSA–Dx precipitated in alum. The most notable effect of CT

Fig. 2. Effect of CT on the primary and secondary antibody responses to native dextran and CSA–Dx. For investigations of TI responses, C57BL/6 mice were immunized with 10 µg native dextran in saline or together with CT adjuvant. For studies of TD responses the mice were immunized with 100 µg CSA–Dx in alum or with CT adjuvant. The mice were bled 10 or 28 days (day 28, for TI dextran only) after primary and 7 days after secondary immunizations. Serum pools from four to seven mice per group were tested in ELISA. Anti-dextran IgM concentrations after challenge with native dextran (A) and anti-dextran IgG concentrations after challenge with CSA–Dx (B) are shown. The data is representative of two similar experiments.
Cholera toxin enhances TI germinal center formation

Fig. 3. Immunization with the TD CSA-Dx conjugate with CT adjuvant generates dextran-specific GC. Frozen splenic sections from C57BL/6 mice were co-stained for the presence of GC with biotin-PNA (developed with avidin–Texas Red) and for the presence of antigen-specific B cells with FITC-Dx. Development GC (A) that are specific for dextran (B) 7 days after secondary immunization with CSA-Dx and CT adjuvant. Magnification ×32.

in the response to CSA–Dx was the observed increase of dextran-specific GC (Fig. 3A and B, and Table 2b).

Collectively, it seems as if CT is able to potentiate splenic GC responses to TI and TD forms of dextran by increasing the number of dextran-specific GC, and possibly by making the GC reaction more efficient, since there is an increase of the secondary humoral response against dextran.

Deposition of dextran in the splenic sections after immunization with dextran

After immunization with dextran, large dextran deposits could be detected in the splenic sections by staining with a biotin-conjugated mAb against dextran. Dextran deposits were detected in sections from mice immunized with native dextran (Fig. 4) as well as with CSA–Dx. The sections from native dextran-immunized mice contained more positive areas and in general the staining was more intense. Staining of serial sections with either PNA or anti-dextran antibody revealed dextran deposits localized within the GC and also in the marginal zone forming a ring around the follicle. It was possible that the poor secondary humoral immune responses obtained following immunization with native dextran were due to retention of large dextran molecules that were unable to be degraded and processed. In order to investigate this, we injected mice with dextranase (an enzyme which breaks down dextran into small subunits) 2 days after primary immunization with native dextran and CT. Upon examination of the splenic sections of the dextranase-treated mice, dextran deposits could not be observed. After a resting period of 4 weeks the mice were immunized a second time with dextran and CT. Neither secondary humoral nor secondary splenic GC responses were affected by this treatment which demonstrates that the persistence of unprocessed dextran was not responsible for the low responsiveness to dextran (data not shown).

Discussion

The humoral response is the end of a long chain of complex cellular migrations and interactions in the intricate process of antigen-induced B cell maturation. The process leading to this maturation takes place mainly in the GC. According to the general belief, the GC reaction is most pronounced in responses to TD antigens. The generation of high-affinity memory B cells has been shown to be absolutely dependent
Cholera toxin enhances TI germinal center formation

Fig. 4. Dextran deposits in the splenic follicles after immunization with native dextran. Frozen sections from C57BL/6 spleens 7 days after secondary immunizations with native dextran administered together with CT adjuvant were stained with a biotin-labeled anti-dextran antibody (revealed by avidin–Texas Red). Results show retention of dextran after native dextran immunization. Magnification ×32.

on T cells although very low numbers of CD4+ T cells are present in the GC (5,25).

Recently Wang et al. demonstrated the formation of dextran-specific GC following primary immunization with native, TI, dextran (3). Other studies have also shown that GC formation occurs after immunization with other TI antigens (4–6). To understand and eventually improve immune responses against TI antigens it is important to compare the mechanisms that are necessary for the generation of a TI and TD immune response. Working with the carbohydrate dextran provides a possibility to simultaneously study a TI and a TD immune response to the same antigen by using dextran either in its native form or low mol. wt dextran conjugated to a protein. In this study we showed that GC formation occurs after immunization with TI dextran approximately to the same extent as observed after immunization with a TD form of dextran. Dextran-specific B cells could be detected in the GC, demonstrated by staining the B cells with FITC-conjugated dextrans as previously described (3). In mice immunized with the TD form of dextran there were only few sites of dextran-binding cells in the spleen and only a minority of the GC were dextran specific. This is to be expected since most of the GC probably are specific for the protein part of the conjugate. Also, in correlation with this, the primary anti-dextran antibody response after immunization with CSA–Dx is of a lower magnitude than after immunization with native dextran.

It has been suggested that B cells activated by TI antigens behave differently than B cells activated by TD antigens (26). Following TD antigen challenge many of the proliferating B cells within the PALS would enter the follicles and initiate a GC reaction, while most B cells proliferating in response to a TI antigen would differentiate into plasma cells outside the follicle. Our results from the primary immunizations with TI and TD forms of dextran diverge from this model, as GC formation occurred to the same extent in both TI and TD responses. It is clear, however, that the TI GC reaction is insufficient since it does not result in the generation of memory B cells. This is probably due to inefficient T cell help and lack of different co-stimulatory signals during the GC reaction. Our findings indicate that it may not be the formation of GC per se that is defective in the response to TI antigens, rather the GC reaction is not very productive in terms of generating a good memory response.

It has been suggested that the formation of GC in response to TI antigens is T cell dependent (4). In agreement with this hypothesis are experiments with athymic mice which indicate that GC formation does not occur after dextran immunization unless T cells are present (E. Sverremark and C. Fernandez, submitted).

We have previously shown that the secondary serum antibody response to native dextran is suppressed compared to the primary response. Unresponsiveness caused by exhaustive proliferation (clonal deletion) is claimed to occur when B cell activation by an immunogenic dose of polysaccharide antigen takes place in the absence of generation of memory cells. The pronounced degree of suppression of the secondary response may be related to poor processing and presentation of the uniform and repeating α(1–6) epitope on dextran (23,24). In correlation with this were the results from mice immunized twice with native dextran. Notably, the dextran-specific B cells had disappeared from the spleen. When dextran was adminis- tered in the presence of an alum adjuvant, many sites of dextran-specific B cells were observed in the sections but they were predominantly located outside the GC. The immune response to dextran persists for an extended time period after antigen challenge and this persistence is further enhanced by the use of alum adjuvant. Therefore, it is possible that the presence of dextran-specific B cells in spleens from mice immunized twice with native dextran in alum reflects events that occurred during the primary response.

However, when CT was co-administered with dextran, the secondary responses were significantly improved. The antibody response was enhanced and the number of dextran-specific GC increased. However, the extra-follicular areas with dextran-specific B cells disappeared. This could
be explained by the clonal exhaustion theory described above but is also in agreement with reports describing that during secondary responses, GC B cells migrate to the bone marrow where differentiation into antibody-producing plasma cells occurs (27,28). The enhanced secondary splenic response to native dextran obtained by co-administration of CT was not merely a prolongation of the primary response since the late primary splenic response was diminished.

The PNA staining in sections from mice receiving CT adjuvant was in general very bright. Interestingly, the up-regulation of the PNA receptor on splenic B cells has recently been shown to be dependent on both antigen stimulation and co-stimulation provided by T cells (29). This could indicate that the adjuvant effect of CT at least in part is mediated by T cells.

In spite of the enhanced splenic response, the antibodies produced after immunization with native dextran and CT were mainly IgM, although the antibody levels increased. Apparently, the formation of dextran-specific GC in the response to native dextran occurs independently of IgG class switching. In correlation with this is the identification of a subset of human tonsil IgD+ B cells which has the phenotypical and functional characteristics of GC B cells but does not undergo isotype switching (30). The authors speculated that this subset could be representative of a GC population generated in responses to bacterial carbohydrate antigens. It is also possible that the lack of T cell-produced cytokines in the response to carbohydrates accounts for the absence of the maturation of the antibody response against dextran.

The GC reaction has been demonstrated to be crucial for the generation of B cell memory and affinity maturation of the humoral response to an antigen. It is interesting to observe that this powerful GC reaction occurs to the same extent for a TI and a TD form of the same antigen, although the outcome of the antibody response is quite different for the two forms. However, it seems as if CT has the capacity to activate B cells that are otherwise suppressed in a secondary response to a TI carbohydrate, by increasing the number of antigen-specific GC, and possibly also potentiates the GC reaction resulting in an enhanced secondary anti-dextran antibody response. Whether this adjuvant effect is mediated via another cell type (i.e. T cells) or by a direct effect on the B cells remains to be shown.

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We thank Dr Nils Lycke (Department of Medical Microbiology and Immunology, University of Göteborg) for helpful discussions and Dr Christian Krog-Jensen (Department of Organic Chemistry, Stockholm University) for preparing the CSA-Dx conjugate. This work was supported by grants from the Swedish Medical Research Council.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CT</td>
<td>cholera toxin</td>
</tr>
<tr>
<td>CSA</td>
<td>chicken serum albumin</td>
</tr>
<tr>
<td>Dx</td>
<td>dextran</td>
</tr>
<tr>
<td>GC</td>
<td>germinal center</td>
</tr>
<tr>
<td>PALS</td>
<td>periaortolar lymphoid sheath</td>
</tr>
<tr>
<td>PNA</td>
<td>peanut agglutinin</td>
</tr>
<tr>
<td>TD</td>
<td>thymus dependent</td>
</tr>
<tr>
<td>TI</td>
<td>thymus independent</td>
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