Available online at www.sciencedirect.com
ScienceDirect
Immunology Letters 113 (2007) 70–75
Immunology Letters
www.elsevier.com/locate/

# The role of Blimp-1 in the GC reaction: Differential expression of Blimp-1 upon immunization with TD and TI antigens

Karin Lindroth, Carmen Fernández *

*Department of Immunology, The Wenner-Gren Institute, Stockholm University, S-106 91 Stockholm, Sweden*

Received 21 May 2007; received in revised form 9 July 2007; accepted 21 July 2007
Available online 23 August 2007

**Abstract**

Humoral responses against thymus-dependent (TD) antigens are characterized by Ig class switch, somatic hypermutations (SHM) and generation of memory. These processes are thought to occur in the specialized environment of the germinal center (GC). Some thymus-independent (TI) antigens, such as native dextran B512 (Dx) can also induce formation of GCs, but the responses do not undergo substantial affinity maturation or induction of memory. Immunization with TI Dx affects later TD responses against the same epitope, reducing Dx specific IgG1. We have studied if the different outcome of the TI- and TD-induced GC reaction is due to differences in plasma cell differentiation. The transcriptional repressor B lymphocyte-induced maturation protein, Blimp-1, was used as a marker for differentiation of plasma cells. We show that TI GCs contain Blimp-1 in early and mature GCs, in contrast to TD-induced GCs which strongly express Blimp-1 only in established GCs. Furthermore, the intensity of the Blimp-1 staining is stronger in TI GCs. In addition, we demonstrate that in TD responses after TI priming the pattern of Blimp-1 expression is a mixture of both TI and TD responses. This is novel evidence since these TD humoral responses against Dx display a TI isotype pattern.
© 2007 Elsevier B.V. All rights reserved.

*Keywords:* B cells; TI antigen; Germinal center

## 1. Introduction

The ultimate effector cell in the humoral immune response is the plasma cell. Depending on the manner of activation, the properties of plasma cells can be very different. While a typical thymus-dependent (TD) response gives rise to plasma cells that have undergone somatic hypermutation (SHM) and Ig class switch, plasma cells in thymus-independent (TI) responses usually lack these features. This could partly be explained by a lack of germinal center (GC) formation in TI responses; since the GC provides a specialized environment in which SHM and class switch occur. However, some TI antigens do induce GC formation, for example the carbohydrate native dextran B512 (Dx) [1,2], but even if immunization with Dx induces GC formation in mice, the major isotype in the response is IgM and there is no substantial affinity maturation or generation of a typical memory response. Nevertheless, a kind of memory is produced after immunization. Mice primed with TI Dx show a reduced IgG1 response when later challenged with a TD form of Dx [3]. This negative effect is long lasting and only directed against Dx and not the protein carrier [3,4]. We have previously studied possible mechanisms for this negative effect of TI priming, and our hypotheses is that it may be caused by a commitment of the activated cells to a pathway were they are forced to differentiate into plasma cells and are made unresponsive to signals inducing switch and memory [5].

The exact stimuli needed to commit a B cell to the plasma cell fate are not known. However, work done the recent years have shown that two transcriptional repressors, Bcl-6 and B lymphocyte-induced maturation protein (Blimp-1), are crucial for directing B cells to the GC and to plasma cell differentiation respectively [6–11]. Blimp-1 is required for terminal differentiation of B cells into plasma cells [9–11] but not for the initiation of plasma cell differentiation [12]. And even if an early *in vitro* study suggested that it specifically promotes TI-induced B cell maturation to plasma cells [13], it has been shown that Blimp-1 is expressed *in vivo* in plasma cells during both TI and TD

*Abbreviations:* BCR, B cell receptor; Blimp-1, B lymphocyte-induced maturation protein; CSA, chicken serum albumin; CT, cholera toxin; Dx, dextran B512; GC, germinal center; NP, nitrophenyl; PNA, peanut agglutinin; SHM, somatic hypermutation; TD, thymus dependent; TI, thymus independent

* Corresponding author. Tel.: +46 8 164599; fax: +46 8 6129542.
*E-mail address:* carmen.fernandez@imun.su.se (C. Fernández).

0165-2478/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2007.07.018

responses, as well as in IgG1 producing plasma cells in secondary TD responses [14]. This study also showed that Blimp-1 is not only expressed in plasma cells but also in a subset of GC B cells that have a partial plasma cell phenotype [14]. GC B cells express Bcl-6 [15,16], a transcriptional repressor that negatively controls the expression of Blimp-1 [17,18]. The blocked Blimp-1 expression in GCs has been hypothesized to be important for a successful GC reaction, making sure that the cells have undergone affinity maturation before they terminally differentiate into plasma cells [17,18].

To understand the difference between TI- and TD-induced GCs, and also shed some light on the negative effect on TD Dx responses after TI priming, we wanted to study the maturation of the responding cells. We investigated if an unproductive GC reaction observed during TI responses was due to a bias towards plasma cell commitment, with the focus on the master regulator of plasma cell differentiation Blimp-1. We show here that the GCs induced by TI antigens express Blimp-1 at earlier stages of GC formation and are more intensely stained than TD GCs. In addition, we show that the difference in Blimp-1 expression in TI- and TD-induced GCs may be independent of T cells.

Even if TI responses usually do not generate a typical immunologic memory, they can still influence later immune responses. In mice primed with TI Dx and later challenged with a TD Dx-protein conjugate, the carbohydrate specific IgG1 is greatly reduced, while the IgM response is similar or increased [3]. Thus, the response against the protein carrier has an isotype pattern that is typical for a TD response, while the response against Dx is similar to a TI immune reaction. Here we demonstrate that GCs in this type of responses show a mixture of TD and TI features with regard to Blimp-1 expression.

## 2. Materials and methods

### 2.1. Antigen, mice and immunizations

Native dextran B512 (TI-antigen) with a mol. wt of $(5–40) \times 10^6$ was obtained from INC Pharmaceuticals (Cleveland, OH, USA). A TD form of dextran was obtained by conjugating dextran with a mol. wt of $10^3$ (3–5 glucose units) to the protein chicken serum albumin (CSA) (Sigma, St. Louis, MO, USA). Dextran was conjugated to hydrazide-CSA via its terminal aldehyde group using reductive amination as described before [16]. DNP-Dx was obtained by conjugating DNP Ne-2,4-DNP-L-Lysine, (Sigma–Aldrich Corp., St. Louis, MO, USA) to Dx with a MW of $2 \times 10^6$ (Pharmacia Fine Chemicals, Uppsala, Sweden) as previously described [19].

Cholera toxin (CT) was obtained from List Biological Laboratories (Campbell, CA, USA). Mice were immunized intraperitoneally (i.p.) with 10 μg of native dextran or with 100 μg of CSA-Dx. Even if TI responses can be induced in the absence of adjuvants, 1 μg CT per dose was administered i.p. together not only with the TD form of dextran but also with the TI antigenic forms. This was done for two main reasons, (a) to have a similar immunization situation for both types of antigens and (b) because as we have described before, CT increases and ameliorates the GC formation following immunization with native dextran without changing its TI properties [2]. For the secondary responses, animals were immunized a second time after a resting period of 3 weeks. Immunization with TI-dextran induces a humoral response and a GC reaction even after primary immunization. In contrast, TD responses are only observed after secondary immunizations [2]. Thus, when TI responses are shown in this work, they are primary responses and TD responses are secondary responses.

Female C57BL/6 and nude mice on a BALB/c genetic background were purchased from MB (Ry, Denmark). The mice were maintained in our animal facilities at Stockholm University and were between 2 and 3 months old at the beginning of the experiment. Since BALB/c mice are low responder for Dx [20], nude mice were immunized with DNP-Dx (50 μg/animal) given together with CT i.p. and the spleens were collected day 5 after immunization. The results shown are from two independent experiments with three animals per group. C57BL/6 mice were divided into three groups. One group getting a single TI immunization with native Dx (10 μg/animal) together with CT i.p., was the group control for TI responses. In a second group, control for TD responses, animals were immunized twice keeping a 3 weeks interval, with the TD antigen, CSA-Dx (100 μg/animal) and CT i.p. In the third group we wanted to compare the influence of TI antigens in posterior TD immunizations. The animals in this last group received a single immunization with native Dx (10 μg/animal) together with CT i.p. followed after a 3 weeks resting period by two immunizations, also with a 3 weeks resting period in between, with CSA-Dx and CT. Spleens were collected days 3, 5 and 9 after last immunization. The time points 3 and 9 days were repeated three times with three animals per group in three independent experiments. The time point at 5 weeks was done only once.

### 2.2. Immunohistochemistry

The spleens from untreated and immunized mice were frozen and stored at −85 °C. Later embedded in Tissue Tek OCT compound (Miles, Elkhart, IN, USA), cryosectioned (6 μm), mounted on slides and stored until use. For staining the slides were dried, fixed in acetone (100% 10 min), dried, washed in PBS (2× 10 min) and blocked with horse-serum (1:20 dilution 1 h) before adding the primary antibodies. The slides were incubated with biotinylated peanut agglutinin (PNA) (Vector Laboratories, Burlingame, CA, USA) (5 μg/ml) and goat-anti-Blimp-1 antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) (4 μg/ml) diluted in PBS 1% FCS for approximately 2 h, they were then washed in PBS (2× 10 min) before a second staining step in which Texas Red avidin D (Vector Laboratories, Burlingame, CA, USA) (5 μg/ml) and swine-anti-goat IgG-FITC (Caltag Laboratories, Burlingame, CA, USA) (1:100) in PBS 1% FCS were incubated on the slides for 2 h. The slides were then washed in PBS 0.05% Tween (2× 10 min), dried and mounted with fluorescent mounting medium (DAKO Corporation, Carpinteria, CA, USA) before examined with a UV-microscope. When irrelevant antibodies were used as isotype control no staining was observed (not shown). All slides were read “blind” and by two people. The photographs were

Download English Version:

https://daneshyari.com/en/article/3356293

Download Persian Version:

https://daneshyari.com/article/3356293

Daneshyari.com