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Maintenance of long-term immunological memory by Ig+CD45R+ non-plasma B cells following mucosal immunizations

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ABSTRACT

To determine whether long-term immunological B cell memory following mucosal vaccinations is maintained by terminally differentiated Ig−CD45R− plasma cells or Ig+CD45R+ B cells, we immunized mice orally with the non-toxic B subunit of cholera toxin (CTB) as a carrier protein haptenated with FITC (CTB-FITC) plus CT adjuvant. We found that the adoptive transfer of Ig+CD45R+ but not the Ig−CD45R− cells, resulted in higher numbers of FITC-specific IgA-secreting cells in the intestine as well as higher anti-FITC serum IgA titers, suggesting that long term B cell immunological memory following oral vaccinations preferentially resided within the Ig+CD45R+ B cell population.

1. Introduction

Several theories have been put forward to explain the mechanism of induction and maintenance of immunological memory, all of which have as their underlying basis the clonal selection theory, set forth by Jerne and further modified by Burnet [1,2]. B cell "memory" has thus been associated with antigen-encounter followed by Ig isotype switch from IgM to IgG or IgA and affinity maturation following extensive proliferation and selection of high affinity clones in germinal centers [3]. Consequently, most studies have addressed the issue of "memory" in the context of IgG or IgA-bearing/producing B cells in peripheral lymphoid tissues either a short time after priming immunizations (acute phase) or after boosting immunizations following a resting period (effector memory phase). Studies on the maintenance of memory in the resting memory phase, when the acute phase of the response (particularly against non-replicating antigens) has completely subsided and in the absence of boosting immunizations, particularly following mucosal immunizations, remain sparse. Maintenance of immunological memory at mucosal surfaces is of particular importance since most pathogens enter the host through mucosal membranes and several studies have demonstrated that local immunological responses are required for local protection in mice and primates [4–8].

In general, oral immunizations with most soluble protein antigens in the absence of adjuvant induce systemic tolerance rather than immunity [9]. Therefore, potent mucosally effective adjuvants in the form of bacterial toxins, their detoxified derivatives and mucosal delivery systems have been used to induce local immune responses to unrelated antigens [9,10]. The model used in this study was established several years ago when it was shown long-term immunological memory to unrelated antigens can be induced and maintained by cholera toxin as adjuvant [11]. Using this model it was also found that long-term hapten-specific (DNP) memory was maintained by IgM in serum and that it appeared that this hapten-specific memory required carrier-specific T cell memory [12,13]. More recently, it was reported that in the resting memory phase following mucosal immunizations with CTB-FITC plus CT adjuvant, FITC-specific IgM-secreting cells in bone marrow, followed by spleen, dominated the response [14]. However, that study was not designed to address more directly the question of whether long-term memory was maintained by Ig+CD45R+ B cells, which includes IgM+ cells, or by Ig−CD45R− cells, which include plasma cells. To address this question, we immunized mice orally with CTB-FITC plus CT adjuvant and following a resting period of 16 months, isolated Ig+CD45R+ and Ig−CD45R− cell populations by magnetic bead sorting and adoptively transferred them to syngeneic recip-

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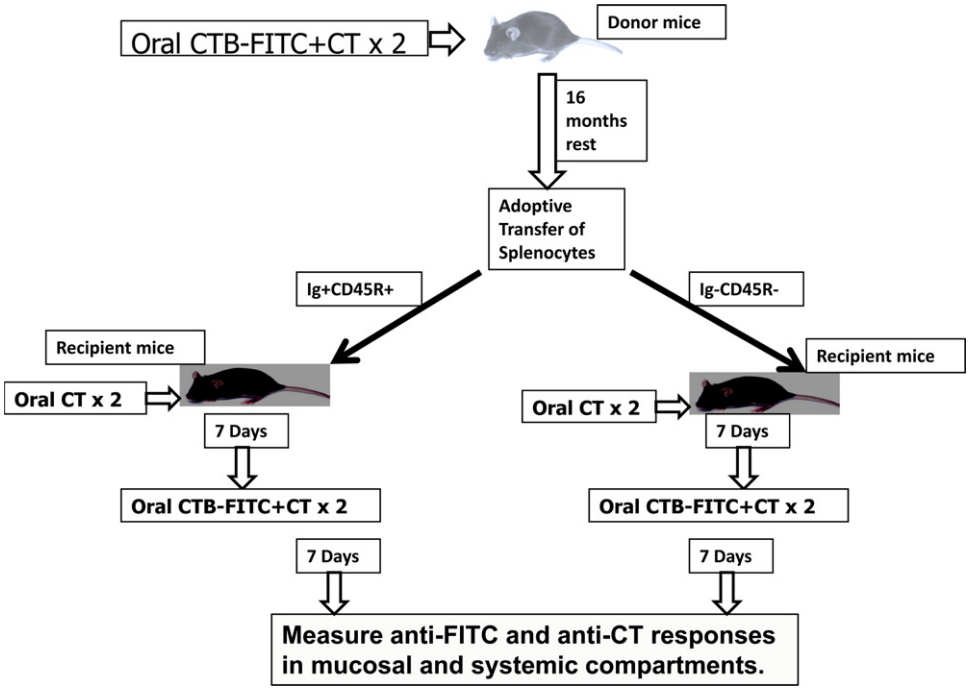

Fig. 1. Protocol for induction of long-term hapten-specific B cell memory and adoptive transfer. Female C57bl/6 mice at the age of 6–8 weeks were immunized orally twice with the non-toxic B subunit of monoganglioside binding moiety of cholera toxin (CT) as a carrier protein, haptenated with FITC (CTB-FITC) plus CT adjuvant. Following a resting period of 16 months, we isolated Ig+CD45R+ and Ig−CD45R− B cell populations from the vaccinated donors and adoptively transferred them to syngeneic host mice primed orally with CT. The host mice were then boosted orally 7 days later with a single dose of the recall vaccine, i.e. CTB-FITC plus CT.

ient mice primed orally with CT and 7 days later boosted the recipients orally with CTB-FITC plus CT. In this model, while the host anti-FITC antibody responses exclusively represented memory-type responses, the host anti-CT antibody responses consisted of a combination of both memory and acute responses.

2. Materials and methods

2.1. Mice and immunizations

Sixty female C57bl/6 mice were purchased from Charles River Breeding Laboratories at the age of 6–8 weeks at the onset of the studies. Oral immunizations were performed with 25 μg CTB-FITC (Sigma/Aldrich) plus 2.5 μg CT (List Biological Laboratories Inc., Campbell, CA) in 3% bicarbonate buffer in a volume of 0.5 ml. Two oral immunizations were performed without anesthesia twice at a 2 weeks interval. A subset of mice was sacrificed 16 months following the final immunization (persistent/resting memory phase), after a single booster immunization with CTB-FITC plus CT. Another subset of mice that were immunized 16 months earlier, were sacrificed and splenocytes were harvested and enriched for Ig+CD45R+ and Ig−CD45R− cells by MACS according to manufacturer's protocol. 10^6–10^7 Ig+CD45R+ or Ig−CD45R− populations were then adoptively transferred intra-venously through the tail vein into groups of 3 individual syngeneic recipient mice that had been primed with two oral immunizations with CT with 2.5 μg per dose at 3 week intervals. The oral priming of the recipient mice served to produce T cell responses against the carrier (CTB). The recipient mice were then given a single oral dose of 10 μg CTB-FITC plus 2.5 μg CT and sacrificed 7 days later and mucosal and systemic antibody responses against both FITC and CT were measured (Fig. 1).

2.2. Sera and tissue collection

Mice were bled through the retro orbital plexus or tails 1 day prior to sacrifice and the sera separated for ELISA. PP were removed from the entire small intestine (between 6 and 12 per mouse) and meshed through a nylon mesh. BM was collected from femur and tibia bones of each mouse by flushing with PBS and re-suspending in complete RPMI-1640 medium. SP were harvested and meshed through a nylon mesh. The tissues were assayed from individual mice with three mice per group. The mice were housed and treated in the vivarium of Novartis Vaccines and Diagnostics Inc. according to guidelines set forth by Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and the United States Department of Agriculture (USDA).

2.3. Magnetic bead enrichment and flow cytometric analysis

Cell suspensions were prepared at 10^7 cells per ml and biotinylated goat anti-mouse IgM antibody (Southern Biotechnology Associates, Alabama) was added at a 1:50 dilution in PBS and incubated at 4 °C for 10 min. Unbound antibody was washed with PBS, the cells were resuspended in staining buffer (provided by MACS kit, Miltenyi) and streptavidin-magnetic microbeads were added at a 1:16 dilution. The cells were incubated at 4 °C for 15 min and washed with the staining buffer. The cells were then run over columns provided by the kit and placed on the magnet and non-adherent cells were collected as the Ig− population. 0.5 ml of staining buffer was run over the column twice and the non-adherent cells were collected and pooled each time with the original non-adherent cells as the total Ig− population. The adherent cells were recovered by removing the column from the magnet and running 1 ml of staining buffer with a plunger and formed the Ig+ population. The cells were counted using an automatic cell counter (Vi-cell XR, Beckman Coulter). To acquire the Ig−CD45R− population, the Ig− population was stained with biotinylated rat anti-CD45R (Beckton Dickenson) and exact same protocol that was used to isolate the Ig+ population was used to isolate the Ig−CD45R− population. The Ig+ and Ig− populations were analyzed by FACS for surface expression of both IgM and CD45R and were shown to be 90% and 1%, respectively (Fig. 2). SP or BM cell sus-