Immunobiology 218 (2013) 10–20

Contents lists available at SciVerse ScienceDirect

Immunobiology

journal homepage: www.elsevier.de/imbio

Induction of ssDNA-binding autoantibody secreting B cell immunity during murine malaria infection is a critical part of the protective immune responses

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ARTICLE INFO

Article history:
Received 10 November 2011
Received in revised form 19 January 2012
Accepted 21 January 2012

Keywords:
Autoantibody
B cell
Immune response
Malaria infection
ssDNA

ABSTRACT

Although it has been hypothesized that autoimmune-like phenomena may play a critical role in the protective immune responses to both human and animal malaria, there are still no evidence-based data to support this view. In this study we demonstrate that the majority of anti-single stranded (ss) DNA autoantibody secreting B cells were confined to $B220^{+}CD21^{+}CD23^{-}$ cells and that these cells expanded significantly in the spleen of C57BL/6 mice infected with *Plasmodium yoelii* 17X non-lethal (*Py*NL). To determine the role of ssDNA-binding autoantibody secreting B cell responses in murine malaria, we conjugated generation 6 (poly) amidoamine dendrimer nanoparticles with ssDNA to deplete ssDNA-binding autoreactive B cells *in vivo*. Our data revealed that 55.5% of mice died after DNA-coated nanoparticle-mediated *in vivo* depletion of ssDNA-specific autoreactive B cells and subsequent challenge using *Py*NL. Adoptive transfer of B cells with ssDNA specificity to mice, followed by *Py*NL infection, caused a later appearance and inhibition of parasitemia. The possible mechanism by which the ssDNA-binding autoantibody secreting B cells is involved in the protection against murine malaria has also been demonstrated.

Introduction

Although T and B lymphocytes with hazardous autoreactive potential are deleted during their maturation, a low level of autoimmunity is still present in all healthy individuals in the form of naturally occurring autoantibodies (aAbs) and autoreactive T and B cells (Erikson et al. 1991; Goodnow et al. 1988; Rose 2002; Russell et al. 1991). Naturally occurring antibodies (Abs) and aAbs show a degree of polyreactivity to pathogens (Zhou et al. 2007). Certain naturally occurring aAbs have been reported to play beneficial roles by directing the immune responses in favor of the host through their immunomodulatory properties (Adelman et al. 2007; Schluter et al. 2003).

On the other hand, bacterial, viral and parasitic infections are known to induce autoimmune-like phenomena. However, controversy remains in regard to whether infections can really induce autoimmune diseases, because most of the accumulated evidence that infections induce autoimmunity come from studies of animal models where diseases have been transferred to naïve animals with autoimmune cells or aAbs from autoimmune model animals (Regner and Lambert 2001). In many instances, autoimmunity has been demonstrated to have a possible role in allowing a rapid immune response to limit the foreign Ag concentrations in the early stages of an infection (Germain et al. 2002; Stefanova et al. 2002).

It has been hypothesized that the autoimmune-like states induced by some infections might be closely associated with the protective immune responses (Baumgarth et al. 2005; Russo and Lopalco 2006). Daniel-Ribeiro et al. hypothesized that aAbs may have protective functions against malarial infections (Daniel-Ribeiro 2000; Daniel-Ribeiro and Zanini 2000). However, evidence has yet to be demonstrated to confirm this hypothesis. To study the role of ssDNA-binding aAb-secreting B cells in malaria, we performed adoptive transfer of B cells with ssDNA specificity into mice, and adopted a novel method to deplete these cells *in vivo*.

The emergence of multidrug resistance and lack of vaccines have made malaria control challenging. Therefore, additional efforts

Abbreviations: ssDNA, single-stranded deoxyribonucleic acid; PAMAM, polyamidoamine; Ab, antibody; aAb, autoantibody; Ag, antigen; ASC, antibody secreting cell.

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doi:10.1016/j.imbio.2012.01.018

should be focused on basic research (Callaway 2007). Since the clinical significance of the co-existence of autoimmunity and malarial infection remains unknown, the present study was undertaken to clarify the role of malaria-induced ssDNA-binding aAb-secreting B cell responses.

Materials and methods

Mice

C57BL/6 (B6) male mice, 10 weeks old, were mainly used to conduct the studies. The mice were maintained under specific pathogen-free conditions. The experiments were performed according to the ethical guidelines established by the University of the Ryukyus for the care and use of experimental animals.

Parasite infection and parasitemia

The infection of mice with a non-lethal (NL) strain of *Plasmodium yoelii* 17X (*Py*) was initiated by intraperitoneal (*i.p.*) injection of 1×10^4 parasitized erythrocytes per mouse. Re-infection (1×10^7 parasitized erythrocytes per mouse) was performed at least after 3 months after recovery. Parasitemia was monitored by oil immersion microscopic examinations of thin blood film stained by Giemsa's solution after methanol fixation.

Single-stranded DNA (ssDNA) ELISA

The serum IgG and IgM Abs reactive with ssDNA were determined by an ELISA kits purchased from Alpha Diagnostic International (San Antonio, USA). All ELISAs were performed according to the manufacturer's instructions. The antibody index for the anti-ssDNA autoantibodies (aAbs) was calculated as follows: (absorbance of the test sample minus absorbance of the negative control, divided by absorbance of the positive control minus absorbance of the negative control) × 100.

Preparation of PyNL Ags

Heparinized blood was collected from *Py*NL-infected mice showing more than 30% parasitemia by cardiac puncture. After 3 washing with an excess of cold PBS, the pellet was resuspended in Eagle's MEM. The cell suspension was then passed through a MEM-equilibrated column packed with fibrous cellulose powder (CF11, Whatman International Ltd., Maidstone, England), and the pure erythrocytes were collected as eluates (Homewood and Neame 1976). The infected erythrocytes were then collected by Percoll gradient centrifugation, and the parasites were released from infected erythrocytes by saponin treatment. Finally, the parasites were subjected repeatedly to rapid freezing at −80 °C and then thawing to elute the *Py*NL Ags.

PyNL ELISA

The *Py*NL ELISA was performed by a commonly used Ag-specific ELISA method with some modifications. The antibody index was calculated according to method described for the ssDNA-binding antibody.

ssDNA ELISPOT assay

The ELISPOT assays were employed to measure the amount of aAb secreting B cells against ssDNA. The PVDF-bottomed-well ELISPOT plates (MultiScreen HTS^{TM}, Millipore Co., MA, USA) were coated with 25 μg of salmon testes ssDNA (Sigma) per well in sterile TBS and incubated overnight at 4 °C. After a blocking step, the plates were washed, and 100 μl of cell suspension containing 5×10^5 or 2×10^5 cells were added to triplicate wells, followed by incubation for 3 h at 37 °C under 5% CO_2. After washing, the plates were filled (100 μl/well) with AP-conjugated goat anti-mouse IgG (Sigma Chemical Co., MD) and were again incubated overnight at 4 °C. Following washing and addition of the BCIP/NBT substrate, and an additional 15–20 min of incubation, the plates were prepared for counting of blue spots microscopically.

PyNL ELISPOT assay

The *Py*NL ELISPOT assay was performed by a method as described for ssDNA ELISPOT except that the coating of the microplates was done with *Py*NL Ag.

Preparation of mononuclear cells from spleen

The spleen was minced, and a single cell suspension was harvested into Eagle's MEM supplemented with 2% heat-inactivated serum by passing the minced pieces of spleen through a 200-gauge stainless steel mesh. The single cell suspension was then centrifuged at 1500 rpm, and the subsequent cell pellet was allowed to undergo erythrocyte lysis with a hemolysing solution. Finally the pure white blood cells were obtained by centrifugation and washing with Eagle's MEM.

Flow cytometry

FITC-labeled anti-B220 (RA3-6B2), PE-labeled anti-CD21 (8D9), and biotinylated anti-CD23 (FcER) (clone 2G8) were purchased from Pharmingen (San Diego, CA) for staining spleen cells. To prevent nonspecific Fc receptor-mediated binding of mAbs, the cells were pre-incubated with anti-CD32/CD16 mAb (2.4G2, BD Pharmingen, San Jose, CA) before staining with the labeled mAbs. The cells were then analyzed by flow cytometry (FACSCalibur; Becton-Dickinson Biosciences, Mountain View, CA).

Purification of splenic $B220^+CD21^+CD23^-$ and $B220^+CD21^-CD23^-$ B lymphocytes

First, total B cells were negatively selected by the depletion of T cells, NK cells and macrophages using a Mini MACS (magnetic activated cell sorting) system (Miltenyi Biotec GmbH, Bergisch-Gladbach, Germany). The splenic $B220^+CD21^+CD23^-$ and $B220^+CD21^-CD23^-$ B lymphocytes were then purified using a cell sorter (FACSAria II, BD Biosciences). The purified cells were used for cell transfer experiments, ELISPOT assays and cytokine assays. For adoptive transfer, 10×10^6 cells per mouse were intravenously injected.

Preparation of the ssDNA–polyamidoamine (PAMAM) dendrimer complex

The generation 6 PAMAM dendrimer (Sigma–Aldrich Inc., MO) was conjugated with salmon testes ssDNA (Sigma Chemical Co., MO) using a modified version of the method described by Bielinska et al. (1999) to prepare ssDNA–dendrimer complexes.

Administration regimen of ssDNA-coated dendrimer nanoparticles

Administration was initiated on day 2 after infection of B6 mice with *Py*NL. A total of 100 μl of DNA-coated nanoparticles containing 1.0 mg of ssDNA was administered to each mouse by *i.p.* injection once every three days. The infected control mice received ssDNA alone or dendrimer alone in the same buffer. Administration was continued until the infected mice succumbed to death or