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Correction of age-associated deficiency in germinal center response by immunization with immune complexes

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Abstract In aging, both primary and secondary antibody responses are impaired. One of the most notable changes in age-associated immune deficiency is the diminished germinal center (GC) reaction. This impaired GC response reduces antibody affinity maturation, decreases memory B cell development, and prevents the establishment of long-term antibody-forming cells in the bone marrow. It is of great importance to explore novel strategy in improving GC response in the elderly. In this study, the efficacy of immunization with immune complexes in overcoming age-associated deficiency in GC response was investigated. We show that the depressed GC response in aged mice can be significantly elevated by immunization with immune complexes. Importantly, there is a significant improvement of B cell memory response and long-lived plasma cells. Our results demonstrate that immune complex immunization may represent a novel strategy to elicit functional GC response in aging, and possibly, to overcome age-related immune deficiency in general.

Introduction

After infection or immunization, aged individuals often generate significantly less antibodies [1], maintain protective titers of serum antibodies for much shorter periods [2], and produce antibodies with affinity lower than that of young controls [3,4]. These deficits are likely to be responsible for increased susceptibility to infection in aged populations. One of the major mechanisms responsible for aged-associated immune dysfunction is the impaired germinal center (GC) pathway of B cell differentiation and maturation. This deficiency in GC reaction in aging leads to diminished antibody affinity maturation, poor memory response, and reduced long-term plasma cells in the bone marrow [5–9]. However, it has been shown that the intrinsic capability of aged B cells to be activated by the initial antigen stimulation is largely intact [7,10]. On the other hand, in primary antibody responses to protein-based antigens, T cell helps for B cell activation and differentiation is a major limiting factor [11]. This limited T help becomes even more profound in old animals [12]. It has been shown that the follicular B cell response was significantly more robust in mice whose T cells were primed with carrier proteins [13]. It is of great importance to identify means to overcome the age-associated GC dysfunction by bypassing the B cell requirement

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for signals from other components of the immune system, such as Th cells.

Fc receptors (FcRs) link the innate and adaptive branches of the immune system and have important functions in the activation and modulation of immune responses. Since both effector cells such as B cell and mast cells, as well as antigen-presenting cells (APCs), such as dendritic cells (DCs), follicular dendritic cells (FDCs), and B cells, express various types of FcRs, immune complex (IC) can exert their immunoregulatory functions by direct signaling effector cells and/or by targeting APCs. Thus, the advantages of IC in inducing immune responses are several folds: ICs can direct activate effector cells; ICs are effectively taken up by professional APCs; and IC-binding to FcR can act as a natural adjuvant and mediate DC maturation. During an immune response, a small amount of IC is trapped on the cell processes of follicular dendritic cells (FDC) in the lymphoid follicles and retained for a long period [14–16], which plays a pivotal role in developing an effective T-dependent antibody response. ICs can stimulate B cells directly and lower the threshold of requirement for T cell help. The BCR affinity threshold for antigen-uptake and presentation is significantly lowered by oligomerization of antigen with antibody [17,18]. ICs increase the avidity of antigen–BCR interaction and enhance the BCR-mediated signals. By fixing complement and bridging BCR with complement, ICs elicit costimulatory signals through co-receptors such as CD19 [18,19]. The co-ligation of BCR and complement receptors (CR) lowers the B-cell activation threshold by 100–1000-folds [19]. In addition, capture of ICs by DCs contributes to an effective T cell response. IC binding leads to many other immunoregulatory and inflammatory responses, including phagocytosis, Ab-dependent cell-mediated cytotoxicity (ADCC), and release of cytokines and other inflammatory mediators central to the protective properties of antibodies [20]. It has been shown that engagement of activating FcγR induces DC activation and maturation [21,22]. FcγR-mediated internalization of ICs by DCs is associated with enhanced presentation of both MHC class I- and II-binding peptides derived from the antigens in the ICs [21,23,24]. The contribution of FcγR and IC to both $CD4^+$ Th cell [25] and $CD8^+$ CTL [22] functions has been demonstrated. These findings underscore the relevance of cross talk through DCs between the B and T cell compartments. The utilization of ICs to enhance the immune responses has been proved effective in inducing tumor immunity [26] and therapeutic vaccination for chronic viral infections such as hepatitis B [27,28].

In the present study, we have analyzed the anti-(4-hydroxy-3-nitrophenyl) acetyl (NP) response in aged mice after immunization with ICs consisting of NP-specific monoclonal antibody and NP-chicken γ-globulin (CGG) conjugate. In mice of the *IgH*^{b} allotype, the antibody response to NP is highly restricted. Most primary NP-specific antibodies bear the λ1 L chain and are encoded by the V_H186.2 gene segment [29]. Our results demonstrate that diminished GC response in aged animals can be significantly restored by IC immunization. In addition, memory response and long-lived plasma cells in the bone marrow were significantly enhanced in aged mice immunized with ICs, indicating a recovery of a functional GC reaction. Our findings suggest that an impaired GC response in aging can be largely overcome by IC immunizations, which may have important clinical implications in designing vaccines and immunization protocols for the elderly population.

Materials and methods

Mice

Young (2–4 months old) and aged (20–24 months old) C57BL/6J (*H-2*^{b}) mice were from Charles River (Wilmington, MA) from cohorts maintained by the National Institute on Aging, NIH. All animals were maintained in autoclaved microisolator cages, and provided with sterile bedding, food, and water. Animal experimentation was performed in accordance with protocols approved by IACUC of Baylor College of Medicine.

Antigens, immune complex formation, and immunization

Hapten NP (Cambridge Research Biochemicals, Cambridge, UK) was conjugated to CGG (Accurate Chemical and Scientific, Westbury, NY) as described [30]. The final conjugation ratio was NP_{22}/CGG. The NP-specific monoclonal antibody (IgG_1) was purified by a protein G kit (Pierce, Rockford, IL) from supernatants of a transfectoma pEV_HCγ1/λ1 [31], which is encoded by germ-line, canonically rearranged V186.2-DFL16.1-JH2 heavy-chain genes and germline λ1 light-chain gene. This IgG1 mAb binds to NP with the dissociation constant $K_d = 1 \cdot 2 \times 10^6$ m. IgG_1 isotype control monoclonal antibody was purchased from Serotec (CRL-1818). Immune complexes or control mixtures were prepared by incubating equal amount of antigen and antibody at 37 °C for 2 h, then at 4 °C for 18 h. For primary immunization, mice were immunized i.p. with a single does of the following preparations: 100 μg NP-CGG in PBS, immune complex containing 100 μg NP-CGG, or 100 μg NP-CGG with isotype control antibody. For secondary immunization, the same injections were given 60 days later.

Immunohistology

Spleens were fresh frozen in OCT embedding media; serial, 6-μm thick frozen sections were cut in a cryostat microtome, thaw mounted onto poly-L-lysine-coated slides, air-dried, fixed in ice-cold acetone for 10 min, and stored at −80 °C [7,32,33]. Immunolabeling of tissue sections was performed as described [7,32,33]. Briefly, splenic GC were labeled by peanut agglutinin (PNA) conjugated to horseradish peroxidase (HRP; E-Y Laboratories, San Mateo, CA) or by biotinylated GL-7 antibody followed by streptavidin-HRP (Southern Biotechnology Associates, Birmingham, AL). Bound HRP activity was then visualized using 3-aminoethyl carbazol as previously described [7]. The splenic GC volume formed after immunization was determined planometrically on photographs of splenic sections as described [34].

Measurement of antibody-forming cells (AFC)

The frequencies of NP-specific AFC from both splenocytes and bone marrow (BM) cells were estimated by ELISPOT assay