Increased Immunogenicity of Mast Cell-Associated Antigens

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D. Skokos[1], H.G. Botros[1], C. Demeure[1], J. Morin[2], R. Peronet[1], G. Birkenmeier[3], S. Boudaly[2], and S. Mécheri[1]

[1]Unité d'Immuno-allergie, Institut Pasteur, Paris, France, [2]Laboratoire d'Immunologie, Génétique et traitement des maladies métaboliques et du diabète, Inserm U 561, Hôpital Saint-Vincent-de-Paul, Paris, France, [3]Institute of Biochemistry, University of Leipzig, Germany

Summary: *Background:* Mouse bone marrow mast cells (BMMC) have been shown to induce lymphocyte activation through the release of membrane vesicles called exosomes stored in intracytoplasmic granules. The aim of this study is to characterize further the immunostimulatory properties of these exosomes *in vivo*. *Methods:* Exosomes isolated from BMMC loaded with bovine serum albumin (BSA) were injected into mice in the absence of adjuvants and antigen-specific antibody responses were measured. *Results:* Exosomes are located in the endocytic pathway since BSA, under its native as well as degraded form, was found to be associated with these compartments. Exosomes were highly efficient in inducing primary and secondary IgG1 and IgG2a antibody responses, suggesting that naturally occurring adjuvants are present in these compartments. Among several routes of immunization tested, intradermal and subcutaneous injections were found to be the most efficient. *Conclusion:* This new mechanism may account for a potential role of MC in the monitoring of tissue environment for the presence of non-self-antigens and in the development of specific immunity.

Keywords: mast cells, exosomes, immune response

Introduction

Beside the classical soluble mediators, mast cell (MC) granules have recently been shown to harbor membrane vesicles termed exosomes which can be released following cytokine- [1] or IgE-mediated MC activation [2] and which are endowed with immunostimulatory activity [1]. Recent results have shown that exosomes may be implicated in the transfer of molecular components from one cell to another. For example, MHC-class II containing exosomes of B cell origin are present in abundance on the cell surface of follicular dendritic cells (DCs) of human tonsil tissue *in vivo,* which themselves do not express these molecules [3]. This prompted us to explore whether MC exosomes act as adjuvants on immune responses against exosome-associated antigens. Here we demonstrate that MC-derived exosomes were highly efficient, in the absence of conventional adjuvants, in inducing antigen-specific IgG1 and IgG2a antibody responses *in vivo*.

Because MC are known to play a prominent role in host defense mechanisms, MC-derived exosomes may participate in the early phases of specific and nonspecific immune responses by monitoring the environment.

Materials and Methods

- *Mice:* DBA/2 and C3H/HeJ mice (6–8-weeks-old) were purchased from Janvier

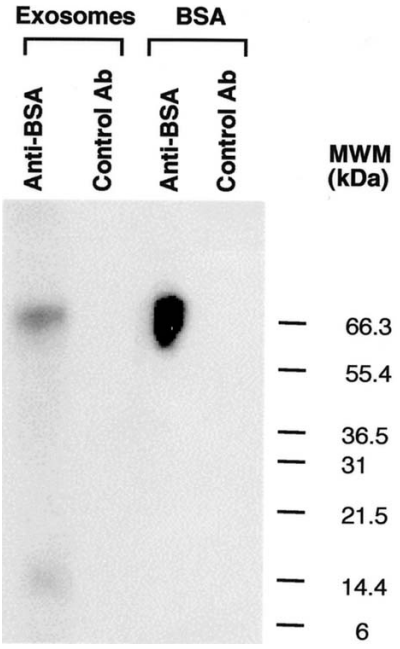

Figure 1. **Exosomes are a site of accumulation of internalized antigens. Exosomes were purified from BMMC incubated with BSA (100 μg/ml) for 24 h. Purified exosomes (15 μg) were separated onto 12% SDS-PAGE, and immunobloted with Abs to BSA and control Abs. Pure BSA (1 μg) loaded onto the same gel was used as control. Results are representative of two experiments.**

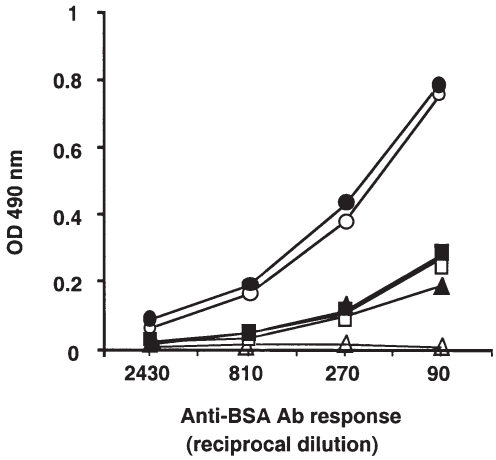

Figure 2. **Induction of antigen-specific immune response by exosomes is dependent on the route of immunization. Exosomes purified from BMMC (DBA/2 mice) cultured for 24 h with BSA (100 μg/ml) were injected SC (●), ID (○), IP (■), or IV (▲) into syngenic mice. The actual amount of BSA associated with 5 mg of exosomes injected per mouse was 50 ng. Controls consisting of 50 ng of BSA in PBS (□) or PBS alone (Δ) were represented. Sera were collected one week after a series of two injections performed at 2 weeks intervals and BSA-specific IgG1 and IgG2a Abs were measured by ELISA. Results are representative of two experiments.**

(Laval, France). All animal care and experimentation was conducted in agreement with the Pasteur Institute animal care and use committee guidelines.

- *Reagents and antibodies:* Bovine serum albumin (BSA) and rabbit anti-BSA Ab were purchased from Sigma (St. Louis, MO). HRP-labeled anti-mouse and anti-rabbit IgG were purchased from Dako A/S, Denmark. Mouse recombinant IL-3 was purchased from BioSys (Compiegne, France).
- *Preparation of MC and exosomes:* Bone marrow-derived mouse MCs (BMMC) were prepared as described by Razin [4] and modified by us [5]. Exosomes were prepared from the supernatant of a 3 week-old BMMC culture as previously reported [1].
- *Protein analysis by SDS-PAGE and western blotting:* To assess the presence of internalized BSA within exosomes, the latter were purified from BMMC (10^6/ml,) preincubated overnight with 100 μg/ml BSA, and subjected to SDS-PAGE analysis.

Results and Discussion

Several cell types including reticulocytes, platelets, B lymphocytes, DCs, and intestinal epithelial cells, have been described to produce exosomes in which functions differ from one cell type to another. Since MC have recently been reported to harbor exosomes in their intracytoplasmic granules [1, 2], we investigated their possible implication in modulating specific immune responses. To provide biochemical evidence that exosomes are

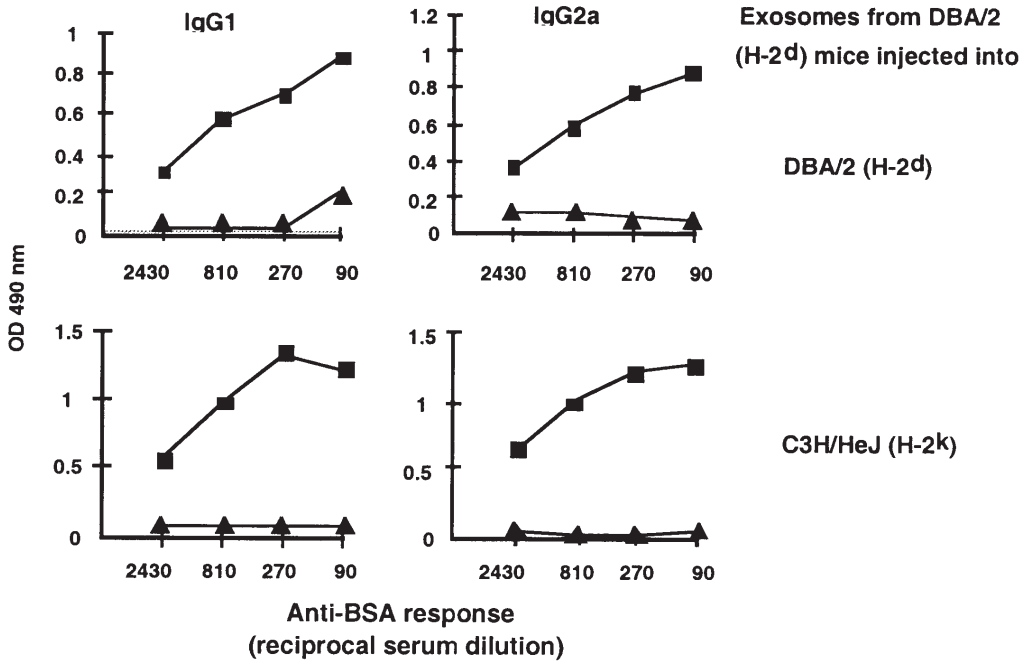

Figure 3. **Exosome-induced antigen-specific antibody response in mice in not restricted to the MHC II haplotype. Experiments were performed as in Figure 2 except that exosomes purified from BMMC (DBA/2 mice) and cultured for 24 h with BSA (100 μg/ml) were injected ID (B) into syngenic mice or into allogeneic C3H mice. Control mice were injected with exosomes alone (H). Results are representative of two experiments.**

located in the endocytic route, we examined whether exosomes harbor exogenous proteins such as BSA internalized by BMMCs through fluid phase. As shown in Figure 1, immunoblot analysis of exosome extract separated by SDS-PAGE demonstrates the presence of BSA. These results confirm previously reported electron microscopy data where immunogold-labeled BSA was found to be associated with exosomes rapidly after internalization [2]. In addition, BSA was found in native and processed form as indicated by the presence of bands with low molecular weight. The colocalization within MC exosomes of exogenous antigens with molecules with immunomodulatory potential such as MHC II [1, 2], CD80, CD86, and adhesion molecules [1] prompted us to examine whether exosomes could act as adjuvants for immune responses against BSA. To this aim, DBA/2 mice were immunized, in the absence of conventional adjuvants, with exosomes isolated from syngeneic BMMC which have been loaded with BSA. Figure 2 shows that exosome-associated BSA (50 ng for 5 μg of exosomes) was extremely efficient in eliciting both IgG1 and IgG2a Abs (not shown), whereas no Ab response could be observed when the same dose of BSA (50 ng) was administered in the presence of PBS or adsorbed on alum (not shown). Among the variety of immunization routes, we found that subcutaneous (SC) and intradermal (ID) delivery of exosomes were the most effective, whereas introduction of exosomes intraperitoneally or intravenously were less efficient (Figure 2). The potent immune response obtained when exosomes were administered ID or SC suggests that these routes of immunization may preferentially target skin DCs, known as the most effective antigen presenting cells to activate naive T-cells. We, indeed, recently demonstrated that MC-derived exosomes were able to induce phenotypic and functional maturation of DC *in vitro* and *in vivo* (unpublished data).

To investigate whether the immunostimulatory potential of exosomes is related to the presence of MHC II molecules, we performed experiments where exosome-associated BSA from H-2^d mice were injected into either syngeneic H-2^d BALB/c mice or allogenic H-2^k C3H mice. The data shown in Figure 3 indicate that anti-BSA antibody responses develop across the MHC haplotype barrier, suggesting that other components associated with exosomes provide their immunostimulatory activity in an MHC II-independent way. A family of proteins which play a role as chaperone for polypeptides and contribute to antigen presentation in various APCs are heat shock proteins (hsps). We recently examined the presence of hsps in MC-derived exosomes and found two hsps, hsp 60 and hsc70 (unpublished data). At this point, we believe that the presence of these hsps within exosomes along with processed antigens accounts for the immunostimulatory potential of exosomes.

The access of exogenous antigens to MC exosomes, which can be transferred to other immune cells, may account for a mechanism by which MC might modulate antigen-specific immune responses.

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S. Mécheri

Immuno-Allergy Unit, Institut Pasteur, 25, rue du Docteur Roux, F-75724 Paris, Cedex 15, France, Tel. +33 1 4568-8245, Fax +33 1 4061-3160, E-mail smecheri@pasteur.fr