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MHC Class II+ Exosomes in Plasma Suppress Inflammation in an Antigen-Specific and Fas Ligand/Fas-Dependent Manner

Seon Hee Kim,* Nicole R. Bianco,† William J. Shufesky,* Adrian E. Morelli,‡ and Paul D. Robbins*‡

Exosomes are small membrane vesicles, ~50–100 nm in diameter, that are formed within the multivesicular bodies of the late endocytic compartment of hemopoietic and nonhemopoietic cells. Exosomes are then secreted into the extracellular space by fusion of the limiting membrane of multivesicular bodies with the plasma membrane. The most studied origins of these nanovesicles are maturing reticulocytes, leukocytes, epithelial, and tumor cells, although many other cell types also secrete exosomes. Exosomes have a characteristic buoyant density and contain a discrete set of proteins, which makes them distinguishable from vesicles released by apoptotic cells (1). Although the exact mechanism of protein sorting into exosomes is poorly defined, it may involve ubiquitination and the ESCRT complex (2, 3). Exosomes originating from B cells, T cells, dendritic cells (DC), and mast cells can confer immunoregulatory signals between cells in either an immunostimulatory or suppressive manner. Indeed, exosomes derived from leukocytes contain many of the important regulatory molecules to carry out this function, such as MHC class I and II, CD80, and CD86, as well as various adhesion molecules that may target exosomes to their acceptor cells (4).

In murine models, exosomes derived from certain cell types such as APC can be either immunostimulatory or suppressive, depending on the cell type and stage of maturation. Peptide-pulsed DC-derived exosomes elicit a potent antitumor immune response in tumor-bearing mice (5, 6) and have been tested in Phase I human trials (7, 8). Tumor cell-derived exosomes carrying tumor Ag, such as P1A and intracisternal A particle protein, also can confer antitumor effects in mice (9), and Toxoplasma gondii Ag-pulsed DC2.4 cell line-derived exosomes can induce humorally-mediated immunity to the T. gondii parasite (10).

Several reports suggest that exosomes also may be immunosuppressive. In this regard, exosome-like vesicles (termed tolerosomes) produced by rat intestinal epithelial cells cultured in the presence of IFN-γ and the model OVA Ag (digested) were able to induce Ag-specific tolerance after injection into untreated mice (11). In addition, allogenetic exosomes from immature rat bone marrow (BM)-derived DC delayed rejection of heart allografts (12). Moreover, T cells, melanoma cells, and ovarian tumor cells are able to generate exosome-like vesicles expressing Fas ligand (FasL), which induce apoptosis of T cells (13–15). In a recent study, exosomes from murine mammary cancer cells were shown to actually promote tumor growth in mice, most likely by the suppression of NK cell function (16). FasL-containing vesicles originating from the placenta also have been found in the first trimester syncytiotrophoblast and in the serum of pregnant women, which may be responsible for conferring immune privilege in the placenta through the suppression of T cell signaling (17, 18). Previously, we have shown that exosomes derived from immature DC treated with IL-10 produce anti-inflammatory exosomes that suppress the onset of murine collagen-induced arthritis (CIA) and reduce the severity of established arthritis (19). Moreover, DC transfected with an adenoviral vector expressing FasL or IL-4 produce exosomes that suppress inflammation in a murine model of delayed-type hypersensitivity (DTH) and partially reverse established CIA through a MHC class II (MHC II)-dependent, but MHC class I-independent mechanism (20, 21). Interestingly, the...
immunosuppressive effect of the exosomes derived from DC transduced with Ad.IL-4 was partially FasL/Fas dependent.

Recent studies also have focused on identifying and characterizing exosomes in vivo as a way to determine their physiologic role(s) as well as their cellular origin. Exosomes have been found in urine (22), malignant effusions (23), bronchoalveolar lavage (24), and blood plasma (25). These plasma-borne exosomes are heterogeneous, being derived from a variety of different cells types including APC. Thus, we were interested in determining whether exosomes derived from the plasma of mice would have an immunomodulatory effect similar to DC-derived exosomes. In this study, we demonstrate that intradermal (i.d.) footpad administration of mouse plasma and serum-derived exosomes is anti-inflammatory in a murine DTH footpad model, but only if derived from the plasma of mice immunized to a specific Ag. The subset of plasma-derived exosomes able to confer immunosuppression were MHC II⁺, FasL⁺, and CD11b⁺. Interestingly, the suppression of the DTH response by the plasma-derived vesicles was dependent upon the presence of FasL in the exosomes and Fas in the recipient, similar to our previous results with BM-DC-derived exosomes. Moreover, the immunosuppressive activity of the plasma-derived exosomes peaked at 7–14 days after Ag immunization. Taken together, these results suggest that the immune response to a foreign Ag is regulated by exosomes in the plasma, produced by MHC II⁺ and CD11b⁺ cells, that have the ability to suppress the immune response in an Ag-specific and FasL/Fas-dependent manner. We hypothesize that the MHC II⁺ and CD11b⁺ vesicles in the plasma serve to dampen the Ag-specific immune response and could play a role in preventing autoimmunity.

Materials and Methods

Mice

Female C57BL/6 (H-2Kb), gld, and lpr mice, all 7–8 wk of age, were purchased from The Jackson Laboratory. The FasL-deficient gld mouse and Fas-deficient lpr mouse have the same genetic background as the C57BL/6 wild-type mouse, expressing H-2Kb and I-Ab. Animals were maintained in a pathogen-free animal facility at the University of Pittsburgh Biotechnology Center (Pittsburgh, PA).

Exosome isolation

Exosomes were isolated from mouse plasma as previously described (19), with minor modifications. Blood was treated with EDTA and centrifuged at 4,000 × g for 5 min and 11,000 × g for 10 min and 20 min to separate the plasma. The plasma was ultracentrifuged at 110,000 × g for 1 h to pellet the exosomes. The exosome pellet was washed in PBS, centrifuged at 110,000 × g for 1 h, and resuspended in PBS. The amount of protein in the exosome preparation was assessed using the Bradford assay (Bio-Rad).

FACS analysis

For flow cytometry, 50–100 μg of exosomes were incubated with a fixed number of 4.5-μm beads (25 μl Dynabeads; Dynal) coated with I-Ab mAb or CD11b mAb. Beads coated with exosomes were labeled with the following PE mAb (BD Pharmingen): H-2Kb, I-Ab, CD11b, CD11c, CD80, or CD11b mAb. Beads coated with exosomes were labeled with 5 nm of colloidal gold (goat anti-mouse or goat anti-rabbit at 1/50; Amersham Biosciences) for 1 h. Grids were washed three times in BSA buffer, three times in PBS, and twice in double-distilled water. The grids were stained with 1% uranyl acetate for 1 min and viewed on a JEM-1011 computer-controlled high-contrast 80 kV transmission electron microscope. No staining was observed when secondary Ab alone was used.

Exosome administration into a DTH model

C57BL/6 mice were sensitized by injecting 100 μg of Ag (KLH or OVA) emulsified 1/1 in CFA at a single dorsal site. Ten days later, one hind footpad of the immunized mouse was injected with 10 μg of exosomes derived from plasma, 24 h before challenge with Ag. The contralateral footpad received an equal volume of saline as an injection control. Mice were challenged in both footpads by injecting 20 μg of Ag dissolved in 20 μl of saline. Footpad swelling was measured with a spring-loaded caliper. Results were expressed as the difference in swelling (×0.01 mm) before and after Ag boost injection.

Statistical analysis

Results were compared using Student’s t test and by ANOVA. Values of p < 0.05 were considered to be statistically significant.

Results

Isolation and characterization of exosome-like vesicles from plasma

We have demonstrated previously that exosomes from immunosuppressive DC suppress the DTH response and ameliorate the severity of CIA in murine models. To investigate the physiologic role of exosomes from endogenous APC in vivo, we examined the plasma of mice for the presence of MHC II⁺ exosomes. Initially, plasma from mice was subjected to differential centrifugation to isolate exosomes. We routinely obtain 10 μg of enriched exosomes from 200 μl of blood (100 μl of serum). The exosome fraction was then negatively stained with phosphotungstic acid and analyzed by electron microscopy (Fig. 1A). The exosome-enriched fraction from plasma contained primarily small cup-shaped vesicles ~50–120 nm in diameter, similar to exosomes produced by DC (19). The plasma-derived vesicles were analyzed by FACS to determine their surface phenotype. In particular, the composition of exosomes from MHC II⁺ cells was analyzed by linking them to Dynabeads coated with I-Ab mAb to immunocapture MHC II⁺ expressing vesicles which then could be labeled with PE mAb and analyzed by FACS (Fig. 1B). The MHC II-containing vesicles were found to contain CD71 and moderate levels of the costimulatory molecules CD80 and CD86. Interestingly, the MHC II⁺ exosomes in the plasma were CD11c⁻, but CD11b⁺, suggesting that they originated from monocyte/macrophages, rather than DC. We also found that all of the MHC II⁺ exosomes were positive for the proapoptotic TNF family member FasL (CD178), but not TRAIL. To examine whether the CD11b⁺ exosomes were also MHC class I⁺ and MHC II⁺, the vesicles were immunocaptured with anti-CD11b mAb-coated Dynabeads. As expected, the CD11b⁺ exosomes were all positive for both MHC class I and MHC II (Fig. 1B).

To confirm that MHC II and FasL were indeed localized on a subset of the plasma exosomes, electron microscopy of immunogold-labeled vesicles was performed. As shown in Fig. 2, a fraction of the plasma-derived vesicles showed strong staining for both MHC II (Fig. 2A) and FasL (Fig. 2B), demonstrated by the amount of 5-nm gold particles on the surface of these vesicles. No labeling was observed when secondary Ab alone was used (Fig. 2C).
Plasma-derived exosomes are immunosuppressive in DTH model

To determine whether the plasma-derived exosomes can regulate the immune response, a DTH footpad model in C57BL/6 mice was used. Exosomes were collected from the plasma of naive mice or from mice that had been immunized with KLH 14 days before. KLH-immunized mice were then injected in the right rear footpad with 10 μg of exosomes from the plasma of either naive or KLH-immunized mice, with the contralateral footpads receiving a saline injection. After 24 h, each footpad was challenged with 20 μg of KLH and footpad swelling was monitored at 48 h after disease induction. As shown in Fig. 3A, the DTH response with saline and exosomes from plasma of naive mice showed an average increase in paw thickness of 0.3 mm. However, exosomes from the plasma of KLH-immunized mice were able to decrease inflammation by >3-fold in both the exosome-injected and saline-injected contralateral footpads.

To demonstrate that the observed immunosuppressive effect was indeed conferred by exosomes, we examined whether the
immunosuppressive effect was dependent upon intact vesicles. We have shown in previous studies that multiple cycles of freeze-thaw or sonication causes the loss of immunosuppressive ability of DC-derived exosomes by disrupting the membrane integrity (19). As shown in Fig. 3B, treatment of plasma-derived exosomes derived from KLH-immunized plasma, 10 μg of exosomes derived from naive (nonimmunized) plasma, or saline control. The mice were then challenged with Ag 24 h later, and footpad swelling was measured after 48 h. B, Suppression of the DTH response by exosomes requires intact vesicles. Ten days after sensitization to KLH, one hind footpad of the immunized mouse (n = 5) was injected with the 100,000 × g supernatant fraction from KLH-immunized plasma, the 100,000 × g pellet (exosome) fraction from KLH-immunized plasma, the exosome fraction from KLH-immunized plasma after three cycles of freeze-thaw, the exosome fraction from KLH-immunized plasma after sonication, the 100,000 × g supernatant fraction from nonimmunized plasma, the exosome fraction from nonimmunized plasma, and saline control. The mice were then challenged with Ag 24 h later, and footpad swelling was measured after 48 h. *, Significance at p < 0.05 in both paws compared with paws of each control.

**FIGURE 3.** Exosomes from KLH-immunized murine plasma are immunosuppressive in the DTH model. A, Suppression of the DTH response by plasma-derived exosomes from preimmunized mice. Groups of mice (n = 5) were sensitized to KLH in CFA on day 0. Ten days later, one hind footpad of the immunized mouse was injected with 10 μg of exosomes derived from KLH-immunized plasma, 10 μg of exosomes derived from naive (nonimmunized) plasma, or saline control. The mice were then challenged with Ag 24 h later, and footpad swelling was measured after 48 h. B, Suppression of the DTH response by exosomes requires intact vesicles. Ten days after sensitization to KLH, one hind footpad of the immunized mouse (n = 5) was injected with the 100,000 × g supernatant fraction from KLH-immunized plasma, the 100,000 × g pellet (exosome) fraction from KLH-immunized plasma, the exosome fraction from KLH-immunized plasma after three cycles of freeze-thaw, the exosome fraction from KLH-immunized plasma after sonication, the 100,000 × g supernatant fraction from nonimmunized plasma, the exosome fraction from nonimmunized plasma, and saline control. The mice were then challenged with Ag 24 h later, and footpad swelling was measured after 48 h. *, Significance at p < 0.05 in both paws compared with paws of each control.

**FIGURE 4.** The anti-inflammatory effects of exosomes in the DTH model are Ag and MHC dependent. A, Anti-inflammatory exosomes are Ag dependent. Groups of mice (n = 5) were sensitized to KLH in CFA on day 0. Ten days later, one hind footpad of the immunized mouse was injected with whole plasma or 10 μg of exosomes from the plasma of KLH- or OVA-immunized mice. Saline was used as a control. The mice were then challenged with KLH 12 h later, and footpad swelling was measured after 48 h (B). Anti-inflammatory exosomes are MHC II dependent. Ten days after mice (n = 5) were sensitized to KLH, one hind footpad of the immunized mouse was injected with whole plasma or 10 μg of exosomes from the plasma of KLH, or exosomes that had been depleted with anti-MHC II beads (Exo-MHC-II depleted), or the exosomes that had bound to the anti-MHC II beads (Exo-MHC II positive). As controls, mice were injected with exosomes depleted with IgG beads (Exo-depletion control) or saline. The mice were then challenged with KLH 12 h later, and footpad swelling was measured after 48 h. *, Significance at p < 0.05 in both paws compared with paws of each control.

Anti-inflammatory exosomes are Ag dependent and MHC II dependent

The results in Fig. 3 demonstrate that the plasma-derived exosomes were only effective against Ag-specific inflammation when derived from the plasma of mice immunized to the Ag. To determine whether the exosomes function to suppress the DTH response in an Ag-dependent manner, exosomes were collected from the plasma of mice that had been immunized with either KLH or OVA Ags. KLH-sensitized mice were then given injections in the right rear footpad with 10 μg of exosomes from the plasma of either KLH- or OVA-immunized mice and each footpad was challenged with KLH. Exosomes from KLH-immunized mice reduced inflammation in both the injected and contralateral paws, whereas exosomes from the OVA-immunized mice had no effect compared with saline alone (Fig. 4A). This result strongly suggests that following i.d. immunization, exosomes in the plasma are able to suppress inflammation in an Ag-specific manner.
To determine whether MHC II+ plasma exosomes indeed are responsible for the Ag-specific effect, the plasma exosome fraction was depleted of MHC II+ vesicles using anti-MHC II paramagnetic beads. After immunodepletion of MHC II-expressing exosomes, the injected exosomes were no longer able to suppress inflammation associated with the DTH response (Fig. 4B). However, activity was restored when the MHC II+ exosomes recovered from the beads were injected into the paws. In contrast, there was no loss of activity when exosomes were depleted with IgG control paramagnetic beads. These data strongly suggest that the in vivo anti-inflammatory effects are conferred by MHC II+ vesicles.

**Fas/FasL signaling is important for anti-inflammatory effect of exosomes**

As shown in Fig. 1B, the MHC II+ serum exosomes contain FasL (CD178). To determine whether the presence of FasL in exosomes and an interaction with Fas is an essential component for the observed immunosuppression, plasma-derived exosomes were isolated from KLH-immunized wild-type or Fas-deficient gld mice and tested for activity in wild-type and in Fas-deficient lpr mice. As shown in Fig. 5, donor exosomes from immunized gld mice were unable to suppress inflammation in the wild-type mice, and donor exosomes from KLH-immunized wild-type mice were unable to suppress inflammation in the lpr mouse. Consistent with these results, donor exosomes from immunized gld mice were also ineffective in the lpr mouse. Taken together, these studies clearly show the importance of the interaction between FasL in the plasma-derived exosomes with Fas in the recipient KLH-sensitized mice to suppress the DTH response, similar to our previous results with BM-DC-derived exosomes (20).

**Time-course analysis of plasma-derived exosomes**

In all of the DTH experiments described above, exosomes from preimmunized mice were collected from plasma 14 days after immunization. To determine when the immunosuppressive activity peaks, a time-course experiment was performed. Plasma-derived exosomes were collected at 0, 1, 3, 7, 14, 28 days after immunization with KLH and tested in the DTH model. Interestingly, the day 7 and in particular the day 14 time points had the highest activity of immunosuppressive exosomes. Moreover, the KLH-immunosuppressive exosomes were detected in the plasma even at 28 days following immunization (Fig. 6).

**Discussion**

Although little is known about the mechanisms through which APC-derived exosomes modulate immunity, the role of exosomes produced by APC seems to be that of a mediator of immune regulatory signals. It is believed that exosomes may transfer proteins, such as Ags, loaded MHC molecules, and costimulatory molecules, between cells of the immune system (26–30). They also may be capable of stimulating, albeit weakly, T and B cells directly through the MHC complex as well as modulating the function of target APC (31–34).

Previously, we have characterized the ability of genetically modified DC-derived exosomes to suppress Ag-specific inflammation and arthritis in mice. Exosomes derived from cultured immune DC, either expressing IL-10, FasL, or IL-4, were shown to reduce paw swelling in the murine DTH model and to suppress the onset and severity of murine CIA (19, 20, 21). Interestingly, in the DTH model, the exosomes suppressed inflammation in both the injected and contralateral footpads, suggesting that local injection of exosomes confers a systemic effect. The immunosuppressive effect also was MHC II dependent, but MHC class I independent.

The majority of the studies to date have examined the composition and function of exosomes derived from cultured APC; therefore, it was unclear whether endogenous APC-derived exosomes have immunoregulatory activity. The results reported here clearly demonstrate that there are MHC II+ exosomes in the plasma with the same morphology as DC-derived exosomes, containing many of the same membrane-associated proteins. However, the MHC II+ plasma-derived exosomes were predominantly CD11c+ in contrast to BM-DC-derived exosomes which are CD11c− (19, 20).

Interestingly, similar to the vesicles from BM-DC, the plasma-derived MHC II+ vesicles were also FasL+. Immunogold labeling of the plasma-derived vesicles confirmed the flow cytometric analysis that a subset of the vesicles was indeed positive for MHC II or FasL. Preliminary flow cytometric analysis using CD81 Ab-conjugated Dynabeads suggests that between 10 and 15% of the serum vesicles are MHC II+ with a similar percentage positive for FasL (data not shown). Although we cannot conclude that all of the MHC II+ vesicles are FasL+, the FACS analysis suggests a significant percentage of the class II-positive vesicles are indeed FasL+. Moreover, our results suggest that the immunosuppressive vesicle is MHC II+ and FasL+.
We also demonstrated that local i.d. injection of the plasma-derived exosomes into the footpad blocks inflammation in the murine DTH model in an Ag-specific manner in the injected as well as the contralateral paw. Moreover, the effect was observed only when the exosomes were derived from the plasma of mice immunized with the same Ag, but not an unrelated Ag or from naive mice. How the Ag specificity is conferred is still unclear, but likely is mediated by exosomes either carrying the Ag or presenting KLH-derived peptides in MHC class I or class II molecules. This result is consistent with a previous report demonstrating that gut processing of orally administered OVA leads to the production of regulatory CD25+ T cells (11, 35). However, these plasma vesicles, reported to be derived from gut epithelial cells, were poorly characterized.

It is important to note that the observed suppressive effects were detected following injection of an exosome-enriched fraction isolated from plasma and serum. Even the exosomes themselves are heterogeneous, with only 10–15% being MHC II+. Although there is still the possibility that the effects are conferred by a factor not associated with MHC II+ exosomes, several lines of evidence suggest that the effect is indeed mediated by intact MHC II+ nanovesicles. In particular, we demonstrated that the suppressive effect in the DTH model is abrogated by sonication or by three cycles of rapid freeze-thaw. In addition, depletion of the exosome-enriched fraction with an MHC II Ab resulted with loss of activity, whereas injection of the vesicles bound to anti-MHC II beads was able to suppress inflammation. Moreover, the effect was dependent upon FasL from the exosome-enriched plasma fraction and Fas in the recipient animals. Taken together, these results suggest that intact, MHC II+, CD11b+, and FasL+ nanovesicles are sufficient to confer Ag-specific suppression of inflammation in the DTH model.

What cell type(s) produce the immunosuppressive plasma exosomes? The analysis of the MHC II+ immunosuppressive exosomes showed that they were CD11c−, in contrast to our results characterizing BM-DC-derived exosomes. Based on protein profile, we believe that the MHC II+ plasma-derived exosomes are likely secreted by monocyte/macrophages, B cells, or possibly from a specific DC lineage at a certain stage of maturation. We have shown that macrophages produce similar exosomes as DC in both morphology and protein content (our unpublished data). However, given that there may be preferential sorting of certain membrane marker proteins into exosomes, the origin of these suppressive exosomes in the plasma is still in question. Although platelets also secrete exosomes, and likely represent a portion of our exosomes, we do not believe they are significant in our experiments since the anti-inflammatory effect requires MHC II+, which is not expressed on platelets.

Our results demonstrated that a FasL-Fas interaction is necessary for the immunosuppressive effect of the MHC II+ cell-derived exosomes in plasma, similar to our results using BM-DC derived exosomes (21). We demonstrated that exosomes isolated from the plasma of FasL-deficient gld mice were ineffective in suppressing the DTH response in wild-type mice. Similarly, plasma-derived exosomes from immunized wild-type mice were ineffective in blocking inflammation in Fas-deficient mice. Since FasL is important for the deletion of mature T and B cells that recognize self-Ag in the periphery, the plasma-derived exosomes may directly affect T and B cell viability (36, 37). Indeed, FasL has been identified in exosomes from various tumor cells and has been proposed to be important for inhibiting the antitumor immune response by killing intratumoral T cells (15, 38–40). It is also possible that FasL+ exosomes are taken up by and alter the function of certain target cells, presumably APC, which subsequently regulate the T cell response. Consistent with this model, we have observed internalization of the labeled, footpad-injected BM-DC-derived exosomes into CD11c+ cells in the footpad and in the draining lymph nodes (21). Also, labeled BM-DC injected i.v. were found to interact with CD11c+ and F4/80+ cells in the spleen and liver. Furthermore, adoptive transfer of CD11c+ cells from mice treated with BM-DC-derived exosomes was able to suppress the DTH response in an Ag-specific manner (21). Thus, we hypothesize that the MHC II+ vesicles isolated from plasma function similarly, interacting with a subset of APC in the spleen and lymph nodes. However, we also have observed that adoptive transfer of CD3+ cells from exosome-treated mice can suppress the Ag-specific immune response. Whether exosomes directly or indirectly stimulate the generation of regulatory T cells is still unclear.

Consistent with the results in Ag-immunized mice, we also have demonstrated that exosomes in plasma can regulate antigen-specific immune responses. Exosomes isolated from plasma of mice with an OVA-expressing s.c. tumor (EG7), but not an OVA-negative parental tumor (EL4), can suppress the OVA-specific DTH response (our unpublished observations). Thus, it is possible that blood-borne nanovesicles play a role in blocking the immune response to tumor Ag, facilitating tumor growth. Inhibition of the activity of the exosomes in plasma could improve the antitumor immune response as well as to other sources of Ag exposure such as viral and bacterial infection. In contrast, enhancement of the activity of the blood-borne vesicles could be useful for treating or preventing autoimmune diseases.

We hypothesize that the functional significance of plasma-borne exosomes is to dampen an active immune response to Ag encountered either through certain routes such as the dermis or the gut. This would keep peripheral self-Ag and commonly encountered Ag (e.g., from food) from causing long-term inflammation and prevent the induction of autoimmunity. In addition, this mechanism could serve to limit the extent of a local immune response. This peripheral suppression by MHC II+ plasma exosomes could limit reactive lymphocytes by anergy, deletion, or even by suppression conferred through induction of regulatory T cells. Because many exosomes are produced per APC, the exosomes in the blood could serve to amplify the suppressive response. This may explain the contralateral effect, treatment of one footpad resulting in a therapeutic effect in the contralateral footpad, that we observed with immunosuppressive DC and both the DC- and plasma-derived exosomes. It is important to note that the optimal time point for the presence of Ag-specific suppressive exosomes in the plasma was between 7 and 14 days, which suggests that these vesicles function to suppress the immune response at a late stage of the immune response.

Our results suggest that harvesting, concentrating, and reinjecting the plasma-derived exosomes at the site of inflammation could be an effective and nontoxic therapy against a variety of autoimmune diseases, including rheumatoid arthritis and diabetes. The fact that plasma-derived exosomes appear to work in an Ag-specific manner could make them very attractive compared with many current nonspecific therapies that suffer from the many toxic side effects of globally suppressing the immune response (41). The likelihood that exosomes may already carry autoantigens or present autoantigen-derived epitopes in MHC molecules obviates the need to first identify the autoantigen before designing a therapy.

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Disclosures
The authors have no financial conflict of interest.

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