Contrasting roles of SPARC-related granuloma in bacterial containment and in the induction of anti–Salmonella typhimurium immunity

Gianluca Rotta,1 Gianluca Matteoli,1 Elisa Mazzini,1 Paolo Nuciforo,1 Mario P. Colombo,2 and Maria Rescigno1

1Department of Experimental Oncology, European Institute of Oncology, 20141 Milan, Italy
2Immunotherapy and Gene Therapy Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori, 20133 Milan, Italy

The role of matricellular proteins in bacterial containment and in the induction of pathogen-specific adaptive immune responses is unknown. We studied the function of the matricellular protein secreted protein, acidic and rich in cysteine (SPARC/osteonectin) in the dissemination of locally injected Salmonella typhimurium and in the subsequent immune response. We show that SPARC was required for the development of organized acute inflammatory reactions with granuloma-like (GL) features and for the control of bacterial spreading to draining lymph nodes (DLNs). However, SPARC-related GL also inhibited dendritic cell (DC) migration to the DLNs and limited the development of adaptive immune response, thus conferring increased susceptibility to the pathogen. In SPARC-deficient mice, both DC migration and antigen-specific responses were restored against bacteria, leading to protective anti-S. typhimurium immunity. This highlights a new function of matricellular proteins in bacterial infection and suggests that initial containment of bacteria can have drawbacks.
S. typhimurium leads to the generation of an acute inflammatory reaction, which is consistent with a granuloma-like reaction (GLR) at the site of infection caused by the recruitment of inflammatory cells (11). This blocks the migration of DCs to the draining LN (DLN) and the subsequent generation of an adaptive immune response.

A characteristic of granulomatous disorders is the increased deposition of several extracellular matrix (ECM) proteins (12), but the role of the ECM in pathogen-induced granulomatous reactions still remains to be elucidated. Functional studies of unspecific inhibition of matrix metalloproteinase (MMP) have shown that MMP plays a role in facilitating dissemination of M. tuberculosis, likely via ECM degradation (13), suggesting a possible role for ECM in forming physical containment of the bacteria. The ECM is involved in tissue scaffolding and remodeling, as well as cell migration, proliferation, and differentiation. The ECM is composed of several structural proteins, growth factors, proteoglycans, and matricellular proteins. The latter is a growing family that comprises tenascin C, which accumulates in granulomas of the lung (14); thrombospondin 1 (TSP1) and TSP2; osteopontin; and secreted protein, acidic and rich in cysteine (SPARC/osteonectin). SPARC is evolutionarily highly conserved and participates in numerous physiological processes (15). It is involved in bone mineralization (16), cell proliferation and migration (17–20), tissue remodeling (21), and angiogenesis (22). SPARC-deficient mice have 50% less collagen deposition (23), which correlates with increased DC migration (24). After enhanced DC migration, the induction of a delayed type hypersensitivity and cutaneous contact hypersensitivity are faster because of a less structured ECM (24). Further, upon skin injury, SPARC is increased in the skin (23). Because massive collagen deposition is crucial at the onset of granulomatous inflammatory reaction (25), we sought to find out whether SPARC-deficient mice had defects in the development of granulomatous reactions and in the initiation of immune response to intracellular bacteria.

In this article, we have characterized the formation of a GL acute inflammatory reaction in response to intradermal injection of S. typhimurium in SPARC-deficient and +/+ mice. In the absence of SPARC, mice failed to develop an organized GL reaction in response to S. typhimurium, leading to an accelerated spreading of the bacteria to the DLN and an increased immune response to S. typhimurium. However, this correlated with decreased systemic spreading of S. typhimurium and conferred higher resistance to the pathogens. These findings highlight a new function of matricellular proteins in bacterial infection and suggest that initial containment of bacteria could be detrimental to the host by limiting the induction of adaptive immune responses.

RESULTS

SPARC−/− mice do not develop organized GL reactions

I.v. injection of S. typhimurium in mice induces the generation of granulomas in the liver that are aimed at bacterial containment (8). We recently showed that intradermal (i.d.) S. typhimurium injection also leads to the induction of an acute inflammatory response in the skin that is reminiscent of a granulomatous reaction that blocks the migration of DCs, as well as of bacteria from the site of injection (11). This system would give us the unique opportunity to analyze factors or molecules involved in organized inflammatory structures and S. typhimurium containment after the dissemination of bacteria from the defined site of injection. An oral or an intravenous injection would lead to diffused spreading of the bacteria and would not allow us to follow their path in a precise way; an intradermal ear injection, on the contrary, allows us to follow bacterial dissemination to a single DLN. The first question that we asked was whether the matricellular protein SPARC played any role in the development of bacteria-induced GLRs. SPARC-deficient and control WT mice were injected in the ear pinna with 10⁷ CFU of attenuated S. typhimurium SL3261 AT. This strain is deficient in the AraA gene and is dependent on p-aminobenzoic acid and 2,3-dihydroxybenzoate for synthesis of aromatic amino acids and growth. The availability of these compounds is limited in mammalian cells, thus restricting the growth of the bacteria (26). At different times, animals were killed, and ears were snap frozen in optimal cutting compound (OCT), sectioned, and stained with various markers to investigate the structure and the composition of the infiltrate.

To be sure that the analyzed sections were effectively representative of the site of inflammation, a minimal dose of FITC-conjugated inert microbeads was cojected with bacteria, and each slide was examined under the fluorescent microscope for the presence of emitting particles. Immunohistochemical decoration at day 5 after infection showed a clearly defined, compact organized structure in the infiltrate of SPARC+/+ ears; it was particularly rich in CD45+ leukocytes and surrounded by a ring of CD11b+ cells, reminiscent of a granulomatous reaction, even though a fibrotic capsule was not observed, but this was expected for a primary reaction (Fig. 1). In contrast, the same markers in SPARC-deficient mice revealed a massive unorganized infiltrate (Figs. 1 and 2). Further staining with anti–S. typhimurium antibody showed that bacteria were, for the most part (but not totally) contained within the GLR in wt mice, whereas they were spread in a wider area of inflammation in SPARC−/− ears (Fig. 1). The absence of GLR in KO mice corresponded to irregular deposition of collagen IV that, by failing to encapsulate the inflamed area, lead to a more dispersed leukocyte infiltrate, as assessed by immunohistochemical staining (Fig. 2) and by Masson trichromic stain of the inflamed area (Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20071734/DC1). This suggests that SPARC is involved in the formation of an organized inflammatory structure with features of a granuloma, likely via the deposition of collagen fibers.

The composition of the leukocyte infiltrate is comparable in SPARC−/− and +/+ mice

We considered the possibility that the observed structural differences of the inflamed site could be caused by an altered pattern of infiltrating cells in SPARC-deficient mice as compared with WT animals, which, for some reason, could be
No relevant discrepancies were outlined in the inflammation pattern, neither in the recruitment of cell populations nor in the kinetic of the recruitment itself, except for the macroscopic granulomatous formation that was detectable only in WT controls. The absence of organized inflammatory reaction correlates with endogenous DC migration from infected site. Generation of specific immune response consequent to inflammation normally requires DC migration from the periphery to DLNs. Nevertheless, we have previously demonstrated that subcutaneous injection of bacteria blocks DC migration from the site of inflammation to DLNs (11). We observed that impairment of granuloma formation in SPARC-deficient mice is associated with a restored capability of DCs to reach noncompatible with granuloma generation. Thus, we analyzed by flow cytometry the infiltrate composition over time after *S. typhimurium* injection. WT and SPARC-deficient mice were injected i.d. with bacteria, the inflamed area was excised, and constituent cells were analyzed at 48 and 72 h (Fig. 3). In these experiments, fluorescent beads were coinjected with bacteria to spot unequivocally the site of inflammation.

In both strains, F4/80 and Gr1+ cells represented the most abundant cell populations, both in frequency and absolute numbers. At these time points, Gr1+ cells are likely to corresponded to inflammatory monocytes, as they were also positive for MHC class II (IA/IE). Lymphocytes and DCs were present in very low percentages and numbers, the latter ranging from 1 to 6% without significant differences between strains and time.
In our previous studies, we showed that both DC and bacteria spreading to DLNs was impaired in WT mice (11). Thus, to evaluate whether SPARC KO bacteria-treated DCs displayed a faster kinetic of maturation, and whether this was responsible for their capacity to migrate after bacterial injection, we tested their ability to mature in response to S. typhimurium or LPS.

BM-DCs were generated from WT and SPARC-deficient mice, and activated with S. typhimurium or LPS. At different time points, the expression of costimulatory molecules was evidenced (Fig. 4 B). This, together with our previous observation that DCs generated from the two strains displayed similar migratory properties in vitro (24), suggested that the restored ability of DCs to migrate from the injection site to the DLN was likely not dependent on intrinsic differences of the DC population. To test this hypothesis, we evaluated whether tissue- or DC-derived SPARC was important to block DC migration from the infected site. We performed a double adoptive transfer experiment. WT and KO mice received contemporarily contiguous i.d. injections of red and green latex-laden BM-DCs from WT and KO strains, respectively. DC transfers were performed in the presence or absence of 10^7 bacterial CFU (SL 3261 AT), and migration of DCs carrying latex to DLNs was assessed. 3 d after injection, animals were killed, DLNs were collected and teased, and cells were released by treatment with collagenase. Cells were then stained for IA/IE or CD11c, and the entire population was acquired by flow cytometry, to determine the absolute numbers of DCs carrying latex beads in DLNs. To evaluate the effective role of bacteria on DC migration in the two strains, we evaluated the efficiency of DC migration expressed as the ratio of absolute numbers of DCs migrating in the absence versus the presence of bacteria. The efficiency of latex+ DC migration to DLNs after bacterial injection was dramatically and significantly higher in SPARC-/- than +/+ mice (Fig. 4 A). The presence of bacteria in KO mice decreased the efficiency of DC migration of only 20% versus 80% reduction observed in control WT animals. Also, when we analyzed the total numbers of migrating cells to the DLN, we found a striking difference when latex beads were injected together with bacteria in SPARC-/- versus +/+ mice (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20071734/DC1). As expected from our previous data, DCs in the SPARC-/- background migrated better than in the WT background (24). We recently ruled out the possibility that latex beads acquired the ability to reach the DLN in the absence of cellular transport because of the less organized collagen deposition in SPARC KO mice (24). Therefore, the latex+ cells that we find in DLN represent cells migrating from the injection site.

These data suggest that SPARC is essential for the process of blocking DC migration from the S. typhimurium–infected site into the DLN.

Tissue-derived, and not DC-derived, SPARC is responsible for block of DC migration

DCs express SPARC (24), which could play a role in cell differentiation and/or maturation; thus, to evaluate whether SPARC KO bacteria-treated DCs displayed a faster kinetic of maturation, and whether this was responsible for their capacity to migrate after bacterial injection, we tested their ability to mature in response to S. typhimurium or LPS.
The number of proliferating T cells was significantly higher in SPARC−/− mice treated with OVA-expressing recombinant bacteria compared with SPARC+/+ mice, indicating an efficient priming of antigen-specific T cells. OVA-specific T cells underwent five cycles of proliferation in SPARC−/− mice, and barely two cycles in WT mice (Fig. S3, available at http://www.jem.org/cgi/content/full/jem.20071734/DC1). As a control, SPARC+/+ and SPARC−/− animals injected with recombinant bacteria carrying the empty vector displayed no OVA-specific T cell proliferation. Together, these results indicate that in the absence of SPARC, bacteria can be recovered from the DLN, and this correlates with specific T cell activation.

Induction of adaptive immune response protects SPARC−/− mice from S. typhimurium infection.

The presence of bacteria in DLNs could be interpreted both positively and negatively. On one hand, it could allow priming of S. typhimurium–specific T cells and, consequently, the generation of a specific immune response. On the other hand, it could represent the first step of infection spreading.

**Figure 4.** DC migration from infected sites is inhibited by host-derived SPARC. (A) Endogenous DC migration is restored in SPARC−/− mice. 10⁷ fluorescent latex (LX) microspheres/site were injected i.d. in mice in four different sites in the presence or absence of 10⁷ CFU of S. typhimurium. Mice were killed at day 3 after treatment and the total number of Ia/Ie+ cells carrying FITC-labeled latex particles per DLNs was assessed after acquisition of the whole sample. The efficiency of latex+ cell migration (the number of latex+ cells in DLN of mice injected only with latex particles is considered as 100% of migration) to the DLNs is shown. The difference between efficiency of migration of latex+ cells in the presence of bacteria (LX + BT) in SPARC+/+ (white bars) versus SPARC−/− (black bars) mice is highly significant (***, P < 0.0001). This is one experiment representative of three each with three mice per group. (B) BM-DCs from SPARC+/+ and SPARC−/− display the same kinetic of activation in vitro. BM-DCs were generated in GM-CSF–conditioned medium and activated with bacteria at a multiplicity of infection of 10:1 or with 1 μg of LPS. Up-regulation of costimulatory molecules was evaluated by flow cytometry at the indicated time points. One of two similar experiments is shown. (C) DC migration is inhibited by host-derived SPARC. BM-DCs from SPARC+/+ and SPARC−/− were loaded with green and red latex beads (LX), respectively. Mice were injected i.d. with BM-DCs from both strains in the presence or absence of bacteria (BT), and cell migration to the DLNs was assessed 2 d after injection. (left) The mean total efficiency of migration ± the SD of six different mice/group is shown. The difference in DC migration efficiency in the recipient SPARC+/+ mice in the presence of bacteria is not statistically significant (P > 0.05). (right) Representative dot plots showing total migrated cells from single DLNs in the different conditions. Recipient line shows the recipient background where BM-DCs from the two donor strains were injected in the presence or absence of bacteria (BT). (top) Dot plots show the recovery of Red LX laden SPARC−/− BM-DCs; (bottom) dot plots show the recovery of Green LX laden SPARC+/+ BM-DCs.
Therefore, we evaluated whether the aforementioned specific response generated was sufficient to face spreading of infection by assessing the lethality of mice after injection of increasing doses of virulent S. typhimurium. We first tested whether DC migration was also impaired after subcutaneous injection of virulent S. typhimurium, i.e., bacteria able to replicate in vivo (SL1344). As shown in Fig. S4 (available at http://www.jem.org/cgi/content/full/jem.20071734/DC1), both 10^3 and 10^5 CFU of S. typhimurium inhibited DC migration to the DLN, a feature that was restored in SPARC^−/− mice.

We then challenged SPARC^−/− and +/+ mice with increasing doses of live, fully virulent S. typhimurium, ranging from 10^3 to 10^7 CFU (Fig. 6). At higher doses of bacteria, both strains displayed an incomparable mortality rate, suggesting that, at these doses, mice were similarly susceptible to S. typhimurium infection. In contrast, at low dose (10^3 CFU of bacteria), SPARC KO animals were dramatically more resistant when challenged with S. typhimurium, as the majority of mice survived till day 22 in the KO mice, whereas by day 11, all of the WT mice were dead. This indicates that even though SPARC^−/− mice are impaired in their ability to form a GL structure, they do not display increased susceptibility to S. typhimurium infection; instead, at low doses they are more protected.

We hypothesized that the presence of a granuloma, by blocking the initiation of an adaptive immune response, was also impeding the control of the growth of S. typhimurium escaping the skin granulomatous reaction. To test this hypothesis, we evaluated whether the augmented survival of SPARC-deficient mice and the early presence of bacteria in DLNs, was associated with a retarded onset of systemic
infection in these animals, measured as colonization of nondraining lymphoid organs. WT and KO mice received a single i.d. injection of $10^3$ CFU of fully virulent and replicating *S. typhimurium*, and were killed at different time points. Bacterial outgrowth in DLNs, nonDLNs, and the spleen was evaluated by plating serial dilutions of lysed cells (Fig. 7 A). 5 d after i.d. injection, colonies were found only in DLNs, with a significant difference between SPARC$^{+/+}$ and SPARC$^{-/-}$ animals, the latter showing a 10-fold higher number of recovered CFUs. This pattern of DLN colonization was conserved up to 9 d after injection. Conversely, at this time, bacteria had massively invaded the spleen and the nonDLNs of SPARC$^{+/+}$ but not SPARC$^{-/-}$ mice, showing a 100-fold higher bacterial load in WT spleen and a 10-fold higher load in WT LNs versus the corresponding organs of SPARC-deficient animals. At day 12, DLNs of both strains were equally colonized by pathogens, but, again, a significantly higher number of bacteria was still found in the LNs and spleen of WT compared with SPARC$^{-/-}$ animals. The majority of WT mice did not survive day 12, making a comparison at later time points impossible.

The sudden increase of bacteria in nondraining organs in WT animals at day 9 could suggest that within the granulomatous reaction, *S. typhimurium* grows undisturbed because of the inability to initiate a rapid anti-*S. typhimurium* response until the bacterial burden is such to disrupt the granuloma structure. The massive colonization of distal nondraining organs by *S. typhimurium* could be suggestive of a nonlymphatically mediated dissemination.

**Subcutaneous vaccination is protective only in SPARC$^{-/-}$ mice**

Thus, if early dissemination of *S. typhimurium* is required to induce a protective anti-*S. typhimurium* response, we would then expect that only in SPARC$^{-/-}$ mice would a low dose of i.d. injected avirulent *S. typhimurium* vaccinate against the fully virulent strain. Both strains of animals were vaccinated with intradermal injection of $2 \times 10^3$ CFU of attenuated *S. typhimurium* SL3261 AT. 2 wk after immunization, mice were challenged i.v. with a lethal dose of virulent *S. typhimurium* (10$^3$ CFU; SL1344), and survival was assessed. All SPARC$^{+/+}$

![Figure 7](image-url)  

**Figure 7.** SPARC$^{-/-}$ mice display reduced systemic bacterial dissemination. (A) Bacterial dissemination was evaluated in SPARC$^{+/+}$ and SPARC$^{-/-}$ animals injected intradermally with $10^3$ CFU of pathogenic *S. typhimurium*. DLNs, nonDLNs, and spleens were lysed and plated at different time points. Total bacterial count/organ is shown $\pm$ the SD of 12 different mice per group. The difference in bacterial recovery in SPARC$^{+/+}$ and SPARC$^{-/-}$ animals is statistically significant (*, P < 0.01). (B) Intradermal immunization with attenuated *S. typhimurium* can protect SPARC$^{-/-}$ but not SPARC$^{+/+}$ mice from subsequent challenge with pathogenic *S. typhimurium*. SPARC$^{+/+}$ and SPARC$^{-/-}$ mice were vaccinated subcutaneously with $2 \times 10^3$ CFU of attenuated *S. typhimurium* or with PBS as a control. 2 wk after vaccination, all mice were challenged i.v. with $10^3$ CFU of pathogenic *S. typhimurium*. Only SPARC-deficient mice vaccinated with bacteria survive upon challenge with virulent *S. typhimurium*. Kaplan-Meyer survival curves are shown. This is one representative of four different experiments each performed on five mice per group. The difference between the SPARC$^{-/-}$-vaccinated group versus all the others is statistically significant (P < 0.01, Log-Rank).
mice, irrespective of vaccination, and nonvaccinated SPARC$^{-/-}$ animals died between 6 and 15 d after injection (Fig. 7 B). On the contrary, 75% of vaccinated SPARC-deficient mice survived the challenge. These results suggest that the i.d. route of administration is highly inefficient for vaccination purposes, likely because of the inability of DCs to migrate to DLNs and to initiate pathogen-specific responses. In SPARC$^{-/-}$ mice where DCs can migrate to the DLN and initiate an antigen-specific response, the i.d. route of administration allows us to generate protection toward virulent S. typhimurium. This experiment also shows that the two strains of mice are equally susceptible to i.v. administration of S. typhimurium (see nonvaccinated mice), indicating that the differences observed in survival of mice challenged with $10^5$ CFU of pathogens intradermally are not caused by strain-dependent intrinsic resistance to pathogens, but to skin-related differences that are presumably conferred by SPARC.

Altogether, these results suggest that although SPARC-proficient mice control infection spreading at early time points, SPARC$^{-/-}$ mice display a much better outcome because of the initiation of a systemic response to bacterial antigens.

**DISCUSSION**

The role of the microenvironment in the control of the immune function has recently been proposed (28–32), suggesting that the outcome of the immune response does not depend uniquely on the interaction between immune cells and antigens. Rather, environmental tissues provide the immune system with different signals that contribute to modulate its responses, according to specific organ requirements. We used an already established skin inflammatory model to monitor the dissemination and spreading of locally injected S. typhimurium and the subsequent immune response (11). Our experimental model suggests that the requirement of the skin to contain pathogen dissemination overcomes the default “program” of the immune system, which is to allow DC maturation and migration soon after pathogen encounter. This is achieved via tissue remodeling and the formation of an inflammatory reaction reminiscent of a granuloma that limits the spreading of the bacteria, similar to what has been described in the lung in response to M. tuberculosis (3, 4) or in the liver and spleen in response to oral S. typhimurium infection (33).

We show here that a major player for the observed effect is environmental SPARC. Matricellular protein SPARC participates in numerous physiological processes, and SPARC-deficient mice display an irregular and more lapsed deposition of collagen fibers (34). We found that in response to S. typhimurium, mice lacking SPARC have irregular collagen deposition and no signs of an organized inflammatory granulomatous reaction, which is consistent with the notion that massive collagen deposition is required for the onset of granulomatous inflammatory reaction (25).

It has been described that the absence of granuloma leads to an increased susceptibility of animals to pathogens (35). Most of these studies were performed in TNF-deficient mice that fail to develop granulomas in response to Bacillus Calmette–Guerin (36) and are highly susceptible to M. tuberculosis infection (37). However, TNF plays a major role in inflammatory responses and intracellular bacterial clearance by inducing macrophages to produce nitric oxide and chemokines (CCL2, CCL5, CCL9, and CXCL10) involved in the subsequent recruitment of inflammatory cells (38). TNF-deficient mice also show a defective homing of T cells that localize in the pulmonary perivascular and peribronchial region (39). Thus, it is very difficult to ascertain whether the observed increased susceptibility to bacterial infection in these mice is caused by the absence of a granuloma or to the lack of TNF-mediated antibacterial effect.

In our model, flow cytometric analysis of inflammatory cells from mice intradermally injected with S. typhimurium reveals no differences in terms of cell populations or cell numbers between SPARC WT and KO animals. However, the histochemical analysis of the injection site reveals that the inflammatory reaction is not organized in SPARC$^{-/-}$ mice. This observation suggests that the absence of SPARC mainly affects the structure and the organization of the infiltrate, rather than its composition, making this a very valuable model to study the role of acute inflammatory reactions in bacterial infection. We observed, by means of immunohistochemical staining that most, but not all of the injected bacteria were confined inside the GLRs in WT animals, whereas they were largely spread in KO counterparts. This correlated with an increased spreading of S. typhimurium to DLN and to the ability of bacteria-laden DCs to reach the DLN. At later time points, we found that WT but not SPARC$^{-/-}$ mice had uncontrolled proliferation of bacteria in nonDLNs and were more susceptible to S. typhimurium infection, suggesting that initial spreading to DLNs is beneficial to control subsequent bacterial spreading. This correlated with the development of an anti-S. typhimurium response: injection of recombinant S. typhimurium expressing OVA in mice adoptively transferred with DO11.10 CD4 T cells revealed that in the absence of SPARC, OVA-specific T cells proliferated in DLNs, suggesting that a specific response is mounted. Thus, whereas granulomatous reaction is actually effective in an initial containment of bacteria, it is disadvantageous at later time points, as it also impedes the development of a protective immune response to the bacteria and their control after escape from the site of infection. Indeed, at low doses of bacteria, the ability of SPARC-deficient animals to mount a rapid and specific immune response was beneficial, and resulted in an increased survival to pathogenic bacteria. Interestingly, at high doses of injected bacteria, both SPARC$^{-/-}$ and $^{+/+}$ mice underwent rapid bacterial colonization and died. Two nonmutually exclusive possibilities could be envisaged: one in which the induced immune response can better control bacteria dissemination, and thus the absence of an organized granuloma in SPARC$^{-/-}$ mice does not impact on mouse survival; and another, in which the formation of a granuloma could also represent a favorable microenvironment for bacteria replication, protected from a possible secondary specific immune response (4). The sudden dissemination of S. typhimurium in WT animals between day 5 and 9 to nondraining organs
would favor this hypothesis, suggesting that bacteria grow undisturbed in the GLR until the granuloma falls apart. The role of protective immune response initiated by DC migration to the DLN is supported by the finding that i.d. injection with low doses of nonvirulent *S. typhimurium* lead to protective immunity to virulent *S. typhimurium* injected i.v. only in SPARC−/− and not +/+ mice. As nonimmunized strains were both similarly susceptible to *S. typhimurium* injected i.v., we can rule out intrinsic differences in the ability of SPARC−/− mice to resist intradermal *S. typhimurium* injection.

After our previous study on the role of environmental SPARC in DC migration, we show here that ECM also plays a role in DC migration to DLNs during bacterial infection. This is likely caused by the formation of a physical block generated by SPARC-mediated collagen deposition. This finding suggests that ECM can be an obstacle for DC exodus from the tissue, and is in agreement with the observation that ECM degradation by matrix metalloproteases is required for DC migration (40–44). In the absence of SPARC, the more lapsed collagen deposition could create holes in the ECM, rendering it traversable by the DCs. Thus, the inability of DCs to migrate to DLNs for T cell activation, as well as the generation of an environment that is conducive to bacterial replication, could account for the observed increased susceptibility of WT mice to *S. typhimurium* infection. We cannot exclude that SPARC could also play a role in blocking the differentiation of monocytes into DCs in the skin, as we showed that intradermal bacterial injection impedes DC differentiation from monocytes (11). However, our adoptive transfer experiments using DCs from SPARC WT or KO mice revealed a direct effect of environmental SPARC on fully differentiated DCs, suggesting that this effect is already exerted on mature DCs. This is a very rapid event, as it occurs 1 d after infection, which is consistent with active tissue remodeling (11). Notably, soluble antigens released by *S. typhimurium*, such as flagellin, that do not require cellular transport to reach the DLN, rapidly induce an immune response (45), thereby confirming our previous data showing that the presence of *S. typhimurium* does not inhibit the presentation of soluble proteins (11). It remains to be established whether highly expressed cell-associated antigens can also find a preferential route of presentation, possibly via nearby recruited phagocytes that are not involved within the granulomatous reaction, but with a delayed kinetic (45). Either way, it is striking that when bacteria cannot gain access to DLNs during infection in WT animals (11, 45), establishment of a protective anti-*S. typhimurium* response is also lacking.

Thus, our results suggest that during skin bacterial infection, two events occur: on one side, DC differentiation from monocytes is impaired; and on the other side, already differentiated DCs, as well as bacteria, are retained at the site of infection by SPARC-dependent mechanisms. This feature does not seem to be unique to bacteria, as in response to *Leishmania monocytogenes* infection there is also a block of DC migration into the DLN during the first week of infection, which then resolves at later time points (46). It would be interesting to evaluate whether in this case there is also SPARC involvement in parasite containment and whether persistence of *L. monocytogenes* disrupts the ECM mesh in its own interest.

In conclusion, these results tell us that when the skin is accidently injured, e.g., by a cut, and is exposed to environmental microorganisms, the immune system responds with the induction of a vigorous innate response culminating with a granulomatous reaction that is aimed at microbial containment. This response, however, retards the establishment of antibacterial-specific responses and is inefficient in controlling *S. typhimurium*’s growth, thus favoring its dissemination. A possible therapeutic implication of these results is that during intradermal bacterial vaccination, one might consider administering a molecule for ECM degradation to favor DC migration into the DLNs and the subsequent immune response. Notably, an old strategy to heighten vaccination to tuberculosis was to co-administer hyaluronidase with Bacillus Calmette–Guerin (47).

**MATERIALS AND METHODS**

**Mice.** 5–6-wk-old BALB/c mice were purchased from Harlan. DO11.10 OVA-TCR transgenic T cells were provided by D. Lo (Scrapes Research Institute, La Jolla, CA). SPARC KO mice, originally on a mixed 129SV/C57BL/6 background, were backcrossed for 12 generations with BALB/cAnNCrl (Charles River Laboratories) to obtain congenic SPARC KO mice. All experiments were performed in accordance with the guidelines established in the Principles of Laboratory Animal Care (directive 86/609/EEC) and approved by the Italian Ministry of Health.

**Antibodies and flow cytometry.** The following monoclonal antibodies and matched isotype controls were purchased from BD Biosciences: CD4 (L3T4), CD8α (Ly2), CD3ε, IA/IE, CD11b (Mac-1), CD11c (N418), CD45R (B220), Gr1 (Ly-6G). Rat anti-mouse F4/80 was obtained from Calab Laboratories. Pure rabbit *S. typhimurium* antiserum was purchased from ViroStat.

Flow cytometry analysis was performed with a FACS Calibur (CellQuest software; BD Biosciences).

**Bacterial strains and preparation for injections.** The *S. typhimurium* derivative SL3261 AT is a metabolically defective and strain generated from the SL1344 WT according to the methodolgy described by Datsenko and Wanner (48). A recombinant strain of SL3261 AT was generated expressing either the gene coding for GST-OVA fusion (SL-OVA) or with the GST alone (SL-pGEX). 10⁷ CFUs of SL-OVA express nearly 1 μg protein. Single colonies were grown overnight at 37°C in Luria broth (LB; Difco) and re-started the next day at 1:10 of the original volume to reach an OD600 = 0.35, which corresponds to 7 × 10⁷ CFU/ml.

**Mice treatments, skin inflammation induction, and latex migration.** Mice were anesthetized i.p. with Avertin 2.5% and shaved at four sites of the dorsal skin, which were drained either by the inguinal or brachial LNs. 10⁷ CFUs of bacteria were diluted to a final volume of 10 μl PBS and injected i.d. using a Hamilton syringe (Thermo Fisher Scientific). 10⁷ FITC-conjugated latex particles of 1 μm in diameter (Polysciences) were injected as tracers in the presence or absence of bacteria. Migration of IA/IE⁺ or CD11c⁺ cells carrying latex to DLNs was assessed 3 d after treatment. At indicated times, mice were killed, DLNs were collected and teased, and cells were released by treatment with 0.25% collagenase at 37°C for 25 min (Collagenase D; Roche). Cells were stained for IA/IE or CD11c expression. For quantification, the entire population of DLN cells was acquired by cytofluorometry and absolute numbers of CD11c⁺ or IA/IE⁺ cells carrying latex beads were determined per LN. The region of the skin corresponding to the site of injection, as identified by faint visual appearance of green latex beads, was separated and cells were released after collagenase treatment and stained for FACS.
analysis as described in Antibodies and flow cytometry. To visualize the inflammatory granulomatous reaction, SPARC−/− and +/+ mice were injected in the ear pinnas or i.d. with 104 CFU of attenuated S. typhimurium SL3261 AT, and mice were killed 5 d later. The specimens were immersed in OCT and snap-frozen in liquid nitrogen.

**In vitro loading of DCs with latex microspheres and mouse treatment.** Murine DCs were generated from BM of SPARC +/+ and In vitro loading of DCs with latex microspheres and mouse treatment. SPARC +/+ or −/− mice were infected by host-derived SPARC. Fig. S3 shows that restored DC migration correlates with increased T cell proliferation. Fig. S4 shows that endogenous DC migration is also restored in SPARC +/+ mice after injection of fully virulent S. typhimurium. A Supplemental materials and methods is also provided. The online version of this article is available at http://www.jem.org/cgi/content/full/jem.20071734/DC1.

M. Rescigno is supported by grants from the Crohn’s and Colitis Foundation of America, the Italian Association for Cancer Research, and the Italian Ministry of Health (Ricerca Finalizzata). G. Matteoli is a recipient of a Fondazione Italiana Ricerca sul Cancro fellowship.

The authors have no conflicting financial interests.

Submitted: 14 August 2007
Accepted: 6 February 2008

**REFERENCES**

1. Ulrichs, T., and S.H. Kaufmann. 2006. New insights into the function of granulomas in human tuberculosis. J. Pathol. 208:261–269.

2. Flynn, J.L., and J. Chan. 2001. Immunology of tuberculosis. Annu. Rev. Immunol. 19:93–129.

3. Kaufmann, S.H. 2001. How can immunology contribute to the control of tuberculosis? Nat. Rev. Immunol. 1:20–30.

4. Flynn, J.L., and J. Chan. 2005. What’s good for the host is good for the bug. Trends Microbiol. 13:98–102.

5. Mert, A., F. Tabak, R. Ozars, R. Ozturk, H. Aki, and Y. Aktugu. 2004. Typhoid fever as a rare cause of hepatic, splenic, and bone marrow granulomas. Intern. Med. 43:436–439.

6. Nakonecni, I., and H.S. Hsu. 1983. Histopathological study of protective immunity against murine salmonellosis induced by killed vaccine. Infect. Immun. 39:423–430.

7. Hormaeche, C.E., P. Mastroeni, A. Arena, J. Uddin, and H.S. Joycey. 1990. T cells do not mediate the initial suppression of a Salmonella infection in the RES. Immunology. 70:247–250.

8. Mastroeni, P., J.N. Skepper, and C.E. Hormaeche. 1995. Effect of anti-tumor necrosis factor alpha antibodies on histopathology of primary Salmonella infections. Infect. Immun. 63:3674–3682.

9. Everett, P., M. Roberts, and G. Donuag. 1998. Susceptibility to Salmonella typhimurium infection and effectiveness of vaccination in mice deficient in the tumor necrosis factor alpha p55 receptor. Infect. Immun. 66:3355–3364.

10. Mastroeni, P., B. Villarreal-Ramos, and C.E. Hormaeche. 1992. Role of T cells, TNF alpha and IFN gamma in recall of immunity to oral challenge with virulent salmonellae in mice vaccinated with live attenuated aro-Salmonella vaccines. Microb. Pathog. 13:477–491.

11. Rotta, G., E.W. Edwards, S. Sangaletti, C. Bennett, S. Ronzoni, M.P. Colombo, R.M. Stemman, G.J. Randolph, and M. Rescigno. 2003. Lipopolysaccharide or whole bacteria block the conversion of inflammatory monocytes into dendritic cells in vivo. J. Exp. Med. 198:1253–1263.

12. Roman, J., Y.J. Jeon, A. Gal, and R.L. Perez. 1995. Distribution of extracellular matrices, matrix receptors, and transforming growth factor beta 1 in human and experimental lung granulomatous inflammation. Am. J. Med. Sci. 309:124–133.

13. Taylor, J.L., J.M. Hattie, S.A. Dreitz, J.M. Troudt, L.S. Izzo, R.J. Basaraba, I.M. Orme, L.M. Matrisian, and A.A. Izzo. 2006. Role for matrix metalloproteinase 9 in granuloma formation during pulmonary Mycobacterium tuberculosis infection. Infect. Immun. 74:6135–6144.

14. Kaarteenaho-Wiik, R., M. Nill, M. Rotta, and Y. Soini. 2007. Extracellular matrix proteins and myofibroblasts in granulomas of sarcoidosis, atypical mycobacteriosis, and tuberculosis of the lung. Hum. Pathol. 38:147–153.

**JEM**

**SPARC-RELATED GRANULOMA AND IMMUNITY TO BACTERIA | Rotta et al.**
17. Sage, H., R.B. Vernon, S.E. Funk, E.A. Everitt, and J. Angello. 1989. SPARC, a secreted protein associated with cellular proliferation, inhibits its cell spreading in vitro and exhibits Ca++-dependent binding to the extracellular matrix. J. Cell Biol. 109:341–356.

18. Hasselaar, P., and E.H. Sage. 1992. SPARC antagonizes the effect of basic fibroblast growth factor on the migration of bovine aortic endothelial cells. J. Cell. Biochem. 49:272–283.

19. Francki, A., K. Motamed, T.D. McClure, M. Kaya, C. Murri, D.J. Blake, J.G. Carbon, and E.H. Sage. 2003. SPARC regulates cell cycle progression in mesangial cells via its inhibition of IGF-dependent signaling. J. Cell. Biochem. 88:802–811.

20. Schemmann, B.J., J.R. Neil, and W.P. Schemmann. 2003. SPARC inhibits epithelial cell proliferation in part through stimulation of the transforming growth factor-beta-signaling system. Mol. Biol. Cell. 14:3977–3988.

21. Tremble, P.M., T.P. Lane, E.H. Sage, and Z. Werb. 1993. SPARC, a secreted protein associated with morphogenesis and tissue remodeling, induces expression of metalloproteinases in fibroblasts through a novel extracellular matrix-dependent pathway. J. Cell Biol. 121:1433–1444.

22. Kupprion, C., K. Motamed, and E.H. Sage. 1998. SPARC (BM-40, osteonectin) inhibits the mitogenic effect of vascular endothelial growth factor on microvascular endothelial cells. J. Biol. Chem. 273:29635–29640.

23. Bradshaw, A.D., M.J. Reed, and E.H. Sage. 2002. SPARC-null mice exhibit accelerated cutaneous wound closure. J. Histoch. Cytochem. 50:1–10.

24. Sangaletti, S., L. Giootsa, C. Guiducci, G. Rotta, M. Rescigno, A. Stoppacciaro, C. Chiodoni, and M.P. Colombo. 2005. Accelerated dendritic-cell migration and T-cell priming in SPARC-deficient mice. J. Cell Sci. 118:3685–3694.

25. Singh, K.P., H.C. Gerard, A.P. Hudson, and D.L. Boros. 2004. Dynamics of collagen, MMP and TIMP gene expression during the granulomatous, fibrotic process induced by Schistosoma mansoni eggs. Ann. Trop. Med. Parasitol. 98:581–593.

26. Hotiseth, S.K., and B.A. Stocker. 1981. Aromatic-dependent Salmonella typhimurium are non-virulent and effective as live vaccines. Nature. 291:238–239.

27. Randolph, G.J., K. Inaba, D.F. Robbiana, R.M. Steinman, and W.A. Muller. 1999. Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. Immunity. 11:753–761.

28. Zhang, M., H. Tang, Z. Guo, H. An, X. Zhu, W. Song, J. Guo, X. Huang, T. Chen, J. Wang, and X. Cao. 2004. Splenic stroma drives mature dendritic cells to differentiate into regulatory dendritic cells. Nat. Immunol. 5:1124–1133.

29. Svensson, M., A. Maroof, M. Ato, and P.M. Kaye. 2004. Stromal cells direct local differentiation of regulatory dendritic cells. Immunity. 21:805–816.

30. Tang, H., Z. Guo, M. Zhang, J. Wang, G. Chen, and X. Cao. 2006. Endothelial stroma programs hematopoietic stem cells to differentiate into regulatory dendritic cells through IL-10. Blood. 108:1189–1197.

31. Alpian, O., E. Bachelder, E. Isl, H. Arnhette, and P. Matzinger. 2004. ‘Educated’ dendritic cells act as messengers from memory to naive T helper cells. Nat. Immunol. 5:615–622.

32. Rimoldi, M., M. Chiappa, P. Larghi, M. Vulcano, P. Allavena, and M. Rescigno. 2005. Monocyte-derived dendritic cells activated by bacteria or by bacteria-stimulated epithelial cells are functionally different. Blood. 106:2818–2826.

33. Mastromeri, P., and N. Menager. 2003. Development of acquired immunity to Salmonella. J. Med. Microbiol. 52:453–459.

34. Bradshaw, A.D., P. Puolakkainen, J. Dasgupta, J.M. Davidson, T.N. Wight, and E. Helene Sage. 2003. SPARC-null mice display abnormalities in the dermis characterized by decreased collagen fibril diameter and reduced tensile strength. J. Invest. Dermatol. 120:949–955.

35. Dannenberg, A.M. Jr. 1982. Pathogenesis of pulmonary tuberculosis. Am. Rev. Respir. Dis. 125:25–29.

36. Kindler, V., A.P. Sappino, G.E. Grau, P.F. Piguet, and P. Vassalli. 1989. The inducing role of tumor necrosis factor in the development of bacte- ricidal granulomas during BCG infection. Cell. 56:731–740.

37. Flynn, J.L., M.M. Goldstein, J. Chan, K.J. Triebold, K. Pfeffer, C.J. Lowenstein, R. Schreiber, T.W. Mak, and B.R. Bloom. 1995. Tumor necrosis factor-alpha is required in the protective immune response against Mycobacterium tuberculosis in mice. Immunity. 2:561–572.

38. Algood, H.M., P.L. Lin, and J.L. Flynn. 2005. Tumor necrosis factor and chemokine interactions in the formation and maintenance of granulomas in tuberculosis. Immunity. 17:520–528.

39. Algood, H.M., P.L. Lin, and J.L. Flynn. 2005. Tumor necrosis factor and chemokine interactions in the formation and maintenance of granulomas in tuberculosis. Immunity. 17:520–528.

40. Zozulya, A.L., E. Reinke, D.C. Bao, J. Karman, M. Sandor, and Z. Fabry. 2007. Dendritic cell transmigration through brain microvessel endothelium is regulated by MMP-α/β chemokine and matrix metalloproteinases. J. Immunol. 178:520–529.

41. Yang, M.X., X. Qu, B.H. Kong, Q.Q. Shao, B.P. Deng, K.H. Ko, and L. Lu. 2006. Membrane type 1-matrix metalloproteinase is involved in the migration of human monocyte-derived dendritic cells. Immunol. Cell. Biol. 84:557–562.

42. Zhao, W., S. Darmann, Q. Fu, J. Chen, H. Cui, J. Wang, F. Okada, J. Hamada, Y. Hattori, T. Kondo, et al. 2005. Hypoxia suppresses the production of matrix metalloproteinases and the migration of human monocyte-derived dendritic cells. Eur. J. Immunol. 35:3468–3477.

43. Baratelli, F.E., N. Heuze-Vourc’h, K. Krysan, M. Dohadwala, K. Kiedl, S. Sharma, and S.M. Dubinett. 2004. Prostaglandin E2-dependent enhancement of tissue inhibitors of metalloproteinases−1 production limits dendritic cell migration through extracellular matrix. J. Immunol. 173:5458–5466.

44. Osman, M., M. Tortorella, M. Londei, and S. Quaratino. 2002. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases define the migratory characteristics of human monocyte-derived dendritic cells. Immunology. 107:73–82.

45. Ravindran, R., L. Rusch, A. Iwano, M.K. Jenkins, and S.J. McSorley. 2007. CCR6-dependent recruitment of blood phagocytes is necessary for rapid CD4 T cell responses to local bacterial infection. Proc. Natl. Acad. Sci. USA. 104:12075–12080.

46. Leon, B., M. Lopez-Bravo, and C. Ardavin. 2007. Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against Leishmania. Immunity. 26:519–531.

47. Bergvist, S. 1950. Nord. Med. Increased reaction to BCG vaccination caused by hyaluronidase. Clin. Infect. Dis. 43:55–56.

48. Datsenko, K.A., and B.L. Wanner. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. USA. 97:6640–6645.