Platelet, Not Endothelial, P-Selectin Expression Contributes to Generation of Immunity in Cutaneous Contact Hypersensitivity

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Leukocyte extravasation is a prerequisite for host defense and autoimmunity alike. Detailed understanding of the tightly controlled and overlapping sequences of leukocyte extravasation might aid development of novel therapeutic strategies. Leukocyte extravasation is initiated by interaction of selectins with appropriate carbohydrate ligands. Lack of P-selectin expression leads to decreased contact hypersensitivity responses. Yet, it remains unclear if this is due to inhibition of leukocyte extravasation to the skin or due to interference with initial immune activation in lymph nodes. In line with previous data, we here report a decreased contact hypersensitivity response, induced by 2,4-dinitrofluorobenzene (DNFB), in P-selectin-deficient mice. Eliciting an immune reaction towards DNFB in wild-type mice, followed by adoptive transfer to P-selectin-deficient mice, had no impact on inflammatory response in recipients. This was significantly reduced in wild-type recipient mice adoptively transferred with DNFB immunity generated in P-selectin-deficient mice. To investigate if platelet or endothelial P-selectin was involved, mice solely lacking platelet P-selectin expression generated by bone marrow transplantation were used. Adoptive transfer of immunity from wild-type

mice reconstituted with P-selectin-deficient bone marrow led to a decrease of inflammatory response. Comparing this decrease to the one observed using P-selectin-deficient mice, no differences were observed. Our observations indicate that platelet, not endothelial, P-selectin contributes to generation of immunity in DNFB-induced contact hypersensitivity. (Am J Pathol 2010, 176:1339–1345; DOI: 10.2353/ajpath.2010.081100)

Infiltration of leukocytes is a hallmark of inflammation. To reach the affected tissues, leukocytes must leave the bloodstream. This process of leukocyte extravasation requires several distinct steps, occurring in sequence. Leukocyte extravasation is initiated by short-lived interactions of adhesion molecules from the selectin family with appropriate carbohydrate scaffolds displayed by several glycoproteins, leading to tethering and rolling of leukocytes along the endothelial lining of the vasculature. Of the numerous adhesion molecules, endothelial P-selectin, along with E-selectin and vascular cell adhesion molecule (VCAM)-1, initiates and sustains rolling interactions of leukocytes in the skin microvasculature.

In addition to a direct interaction of leukocytes with the endothelium lining the vasculature, platelets are becoming more and more recognized to initiate leukocyte rolling in several organs, including peripheral lymph nodes,¹ atherosclerotic lesions² and skin.³ In detail, platelets may influence leukocyte rolling in numerous ways: **First**, formation of platelet-leukocyte aggregates in the bloodstream leads to activation of bound leukocytes,² leading to an increased avidity of leukocyte integrins,^{4–6} which then may allow rolling interactions through binding of leukocyte VLA-4 to en-

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endothelial VCAM-1.⁷ Second, rolling of activated platelets along the vasculature^{2,3,8,9} allows a deposition of platelet-derived pro-inflammatory substances directly to the endothelial cells, which in turn activates endothelial cells, leading to an increased expression/secretion of adhesion molecules and cytokines.^{10–13} Third, formation of platelet-leukocyte aggregates increases leukocyte rolling in lymph nodes⁹ and the skin.³ In addition, this platelet-mediated leukocyte rolling has been shown to be functionally relevant in lymph nodes, lung and the skin. Impaired extravasation of naïve T lymphocytes into the lymph nodes, and thus diminished generation of immunity, in L-selectin-deficient mice,¹⁴ can be restored by infusion of activated human platelets. Interestingly, infusion of activated platelets had no impact on generation of immunity in wild-type mice.¹⁵ Likewise, pulmonary leukocyte recruitment in platelet-depleted mice was abolished in a model of ovalbumin-induced asthma.¹⁶ Furthermore, in a model of chronically induced contact dermatitis, infusion of wild-type, but not P-selectin-deficient, platelets restored the inflammatory response in mice rendered thrombocytopenic.¹⁷

The latter observation is in line with previous findings, showing an impairment of cutaneous inflammation induced by application of haptens, such as oxazolone, in P-selectin-deficient mice.¹⁸ However, while the work by Tamagawa-Mineoka and colleagues¹⁷ has clearly shown a dependency of leukocyte extravasation on platelet P-selectin expression to the skin, the role of P-selectin in the generation of immunity toward cutaneous applied haptens remained to be elucidated. We therefore investigated the role of P-selectin in the generation of immunity in the model of 2,4-dinitrofluorobenzene (DNFB)-induced cutaneous hypersensitivity.

Materials and Methods

Mice

P-Selectin-deficient (P-sel^{-/-}) mice on a C57BL/6J background (homozygous for the Selp^{tm1Bay} mutation) and control wild-type C57BL/6J mice were obtained from Charles River Germany). L-Selectin-deficient mice on the C57BL/6J genetic background¹⁹ were kindly provided by Dr. R. Hynes (Howard Hughes Medical Institute, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts). All mice were maintained on a 12-hour light–dark cycle at the animal facility of the J. W. Goethe University. Mice were fed acidified drinking water and standard chow *ad libitum*. All protocols were approved by the governmental administration (Darmstadt, Hessen).

Murine Bone-Marrow Transplantation Model

Bone marrow (BM) cells were obtained by flushing the femurs of donor P-selectin-deficient mice in C57BL/6J background (Charles River) as described previously.²⁰ Recipient C57BL/6J mice received lethal irradiation doses at 4 to 6 weeks of age (2×500 cGy), and animals

received i.v. injections containing 2 to 3×10^6 BM cells. BM cells from normal C57BL/6J mice were taken as controls. At 6 weeks post-transplantation, by which time the BM of recipient mice was reconstituted, adoptive transfer experiments were performed. The lack of P-selectin expression in recipients of P-selectin-deficient BM-recipient mice was confirmed by analyzing P-selectin expression on platelet activation.²⁰

Adoptive Transfer of Immunity

Adoptive transfer experiments were performed according to published protocols²¹: Mice were sensitized by painting 75 μ l of DNFB (Sigma) solution (0.5% in acetone/olive oil, 4/1) on the back of the mice on day 0. On day 5, spleens and regional lymph nodes were removed from DNFB-sensitized mice, and single cell suspensions were prepared by homogenizing lymph nodes and spleens from donor mice through a nylon mesh (70 μ m, BD Biosciences). The cell number was adjusted to 2×10^8 /ml, and 200 μ l were injected i.v. into each recipient C57BL/6 mouse, followed by immediate application of 20 μ l DNFB at 0.3% to the right ear of the mice.

Analysis of Ear Swelling

CHS response was determined using a spring-loaded micrometer (Mitutoyo, Neuss, Germany) in a blinded fashion. Ear swelling responses of DNFB-challenged ears were compared with the response of the vehicle-treated ear in sensitized animals and were expressed as centimeters $\times 10^{-3}$ (mean $\pm$ SD).

Induction of Thrombocytopenia

In some experiments induction of thrombocytopenia was performed. For this purpose, mice were injected with either a mixture of monoclonal rat anti-mouse-CD42b (#R300, Emfret Analytics) or isotype control antibody (rat IgG) at a dose of 2.5 μ g/g body weight 2 hours before painting with DNFB and 3 days thereafter. Platelet counts were monitored 2 hours after the initial antibody injection and immediately before performing the adoptive transfer.

H&E Histology Staining and Immunohistology

H&E staining was performed according to standard methods established in our laboratory.²² Severity of inflammation was scored semiquantitatively as previously described.²³ The score ranges from one to four, corresponding to no, weak, moderate, or strong staining, respectively. Sections were also stained for infiltration by CD3, neutrophils, and monocytes/macrophages. Six μ m thick cryostat sections were incubated with an antibody specific for either CD3 (polyclonal rabbit, DakoCytomation, Denmark), neutrophils (clone: 7/4, Serotec, Oxford, UK), monocytes/macrophages (clone: F4/80, Serotec), or isotype control (clone: LO-DNP-11, Serotec). Staining

was visualized by addition of appropriate second step antibodies and Fast Red Substrate Kit (Dako). In analogy to the scoring system used for H&E-stained sections, staining was assigned a score ranging from 1 to 4, corresponding to no, weak, moderate, or strong staining, respectively. All sections were independently scored by two observers blinded for the coding of sections.

Qualitative and Quantitative Analysis of Infiltrating Cells²⁴

Ears were longitudinally separated at the cartilage and incubated in RPMI supplemented with 0.05% DNase (Sigma) and Liberase (Roche) at 37°C for 90 minutes. Digestion was stopped by adding an equal amount of RPMI with 5% fetal calf serum at 4°C. Using appropriate inserts, single ear-specimen were grinded for 7 minutes using Medimachine (BD Biosciences). Thereafter cells were passed through a 70 μ m nylon cell strainer (BD Biosciences), washed and analyzed.

Flow Cytometry

Flow cytometry was performed as described elsewhere.²⁵ For analysis of infiltrating cells following antibodies were used (BD Biosciences): fluorescein isothiocyanate (FITC)-CD4 (clone GK1.5), FITC-CD8 (clone 53-6.7), NK cell-defining FITC-NK1.1 (clone

PK136), and FITC-Gr-1 (clone RB6-8C5) recognizing granulocytes and monocytes. Appropriate isotype control antibodies with a corresponding conjugate were used as negative controls.

Statistics

All data are expressed as mean $\pm$ SD. Evaluation of statistical significance was performed as indicated in the text. A *P* value <0.05 was considered to be statistically significant. Sigma Stat 3.5 (Systat Software) software was used for statistical calculations.

Results

Decreased CHS Response in P-Selectin-Deficient Mice

Wild-type and P-selectin-deficient mice were sensitized with DNFB. After challenge with DNFB, the inflammatory response was analyzed by measurement of ear swelling and leukocyte infiltration. Compared with wild-type mice, lack of P-selectin expression led to a significant reduction of both ear swelling and leukocyte infiltration: In wild-type mice, the average ear swelling response was $10.0 \pm 5.4 \text{ cm} \times 10^{-3}$, compared with $1.1 \pm 4.7 \text{ cm} \times 10^{-3}$ in P-selectin-deficient mice (Figure 1A). Based on a semi-

Figure 1. P-selectin deficiency leads to a diminished inflammatory response in DNFB-induced contact hypersensitivity. **A:** Ear swelling evaluated 24 hours after challenge with DNFB of sensitized wild-type (+/+) or P-selectin-deficient mice (P-sel^{-/-}). Data are from at least eight mice per group; *P* < 0.001, *t*-test. **B:** Representative H&E stained specimen from either wild-type or P-selectin-deficient mice 24 hours after challenge with DNFB. Numbers in the upper right indicate the severity of leukocyte infiltration of all mice investigated as determined by a semiquantitative score (ranging from 1: no infiltration to 4: severe infiltration²³). **C:** Furthermore, a decrease in lymphocyte, neutrophil, and macrophage expression was noted in mice lacking P-selectin expression. In detail, sections were stained with the markers indicated in the figures as outlined in the *Materials and Methods* section. Infiltration of respective leukocyte subsets was scored as detailed above. CD3 expression was significantly lower in P-selectin-deficient (1.8 ± 0.3) compared with wild-type mice (2.8 ± 0.3 , *P* = 0.008). Likewise, a notable lower expression of neutrophils and macrophages was observed in mice lacking P-selectin expression (Neu: 2.6 ± 0.3 (wild-type)/ 2.1 ± 0.1 (P-sel^{-/-}), *P* = 0.04; F4/80: 2.6 ± 0.4 (wild-type)/ 1.3 ± 0.1 (P-sel^{-/-}); *t*-test, *n* = 3 mice per group).

Donor	Recipient	Δ ear swelling (cm $\times 10^{-3}$)	p
+/+	+/+		-
+/+	P-sel ^{-/-}		ns
P-sel ^{-/-}	+/+		< 0.05
L-sel ^{-/-}	+/+		< 0.05
		0 10 20 30 40 50 60 70 80	

Figure 2. P-selectin is required for generation of immunity, but is not involved in extravasation of leukocytes to the skin. Wild-type (+/+), L-selectin-deficient (L-sel^{-/-}), and P-selectin-deficient (P-sel^{-/-}) mice were sensitized with DNFB. Five days after DNFB-exposure, mice were sacrificed and lymphocytes were isolated from peripheral lymph nodes and spleens. Isolated cells were injected i.v. into recipient wild-type mice, which were subsequently exposed to DNFB on the right ear. Twenty-four hours later, ear swelling response and leukocyte infiltration was evaluated in recipient mice. Ear swelling response 24 hours after exposure to DNFB in recipient wild-type mice: As previously described, L-selectin significantly contributes to sensitization in the model of DNFB-induced cutaneous contact hypersensitivity, as L-selectin deficiency leads to reduced rolling interactions of lymphocytes with order I-III venules in lymph nodes. We here however provide evidence that P-selectin also is critically involved in generating immunity toward DNFB. By comparing the relative contribution of L- and P-selectin in this process, the contribution of P-selectin seems more detrimental (analysis of variance on ranks—multiple comparisons according to the Dunn's method).

quantitative score,²³ leukocyte infiltration was reduced from 3.4 ± 0.2 in wild-type mice to 2.7 ± 0.2 in P-selectin-deficient mice (Figure 1B). In line, infiltration with lymphocytes, neutrophils, and macrophages was reduced in mice lacking P-selectin expression (Figure 1C). These data confirmed and expanded previous findings observed in CHS models using other sensitizing agents.^{17,18}

P-Selectin Contributes to Generation of Immunity, but Not to Elicitation of the CHS Response

However, these data do not distinguish between (i) a possible role of P-selectin in the initial process of gener-

ation of antigen-specific immunity, (ii) extravasation to the skin during the effector-phase, or (iii) both phases. We therefore performed adoptive transfer experiments to further differentiate the contribution of P-selectin to cutaneous inflammation. Adoptive transfer of lymph node and spleen cells from DNFB-sensitized wild-type mice into P-selectin-deficient mice showed a (not statistically significant) trend toward an increased ear swelling response. In sharp contrast, adoptive transfer from P-selectin-deficient donor mice into wild-type recipients lead to decreased ear swelling, which can be likewise observed using L-selectin-deficient mice as donors (Figure 2). Detailed characterization of the leukocyte infiltrate in recipients of adoptive transfer experiments revealed a decreased extravasation of CD8⁺ lymphocytes and Gr-1⁺ neutrophils. Extravasation of CD4⁺ cells and NK-cells remained unaffected, with a statistically not significant trend toward higher NK-cells in mice adoptively transferred with cells from P-selectin-deficient mice (Figure 3). These data point toward a contribution of P-selectin in the generation of immunity in DNFB-induced contact hypersensitivity. The observed changes are not due to an altered subset distribution in the adoptively transferred cell population, as the distribution of CD3⁺, CD4⁺, CD8⁺ T cells, neutrophils and macrophages, was identical adoptively transferred cells from either wild-type or P-selectin-deficient mice (Table 1).

Platelet, Not Endothelial P-Selectin Expression Contributes to Generation of Immunity

As P-selectin is expressed on endothelial cells, as well as on (activated) platelets, we next thought to investigate the relative contribution of endothelial versus platelet P-selectin in this process. For this purpose, mice selectively deficient in platelet P-selectin expression were generated using bone marrow transplantation.²⁰ Adoptively transferring immunity generated in wild-type mice transplanted with wild-type bone marrow into wild-type recip-

Figure 3. Reduced ear swelling response in wild-type mice after adoptive transfer of leukocytes from DNFB sensitized P-selectin-deficient mice. 24 hours after adoptive transfer and challenge with DNFB, ears were homogenized. From homogenates total cell number was counted, and subsets of infiltrating leukocytes were evaluated using flow cytometry. As indicated below, total cell number is reduced in the ears of mice, which received lymphocytes from P-selectin-deficient mice. This reduction in infiltration leukocytes is due to decreased numbers of CD8⁺ cells and neutrophils. Data shown is from three mice. Statistical evaluation was performed using the *t*-test.

Table 1. Morphologic Characterization of Cells Used for Adoptive Transfer

	Wild-type mice							
	LN		Spleen		LN		Spleen	
	Solvent	DNFB	Solvent	DNFB	Solvent	DNFB	Solvent	DNFB
CD3	52.0 ± 12.7	48.1 ± 6.7	14.6 ± 4.1	12.8 ± 1.6	51.4 ± 7.6	44.8 ± 4.5	11.8 ± 2.7	10.6 ± 1.0
CD4	30.7 ± 5.9	28.2 ± 3.4	10.4 ± 4.0	8.3 ± 0.5	29.8 ± 1.3	28.0 ± 2.2	8.8 ± 2.1	6.6 ± 2.5
CD8	30.8 ± 6.0	28.2 ± 3.4	9.1 ± 2.5	6.9 ± 0.3	28.7 ± 3.6	24.8 ± 3.6	6.1 ± 0.7	4.9 ± 1.8
NK	5.7 ± 2.1	2.3 ± 1.1	5.2 ± 1.0	2.7 ± 1.3	2.1 ± 1.0	1.8 ± 0.8	1.2 ± 1.3	1.3 ± 1.6
Neu	1.9 ± 0.6	1.5 ± 0.5	3.0 ± 0.8	3.7 ± 1.9	1.9 ± 0.9	0.8 ± 0.2	3.7 ± 0.4	3.8 ± 0.8
F4/80	2.0 ± 0.4	3.0 ± 0.8	1.5 ± 0.5	3.7 ± 1.9	1.9 ± 0.9	1.6 ± 1.1	3.0 ± 0.7	2.8 ± 0.8

Single cell suspensions from peripheral lymph nodes (LN) and spleens were prepared from mice 5 days after painting with DNFB or solvent by gently passing through a nylon mesh. Cells were stained with fluorescently labeled antibodies binding to the respective leukocyte subsets, or appropriate isotype control antibodies. We observed no difference in the leukocyte distribution in peripheral lymph nodes and spleen of wild-type compared with P-selectin-deficient mice. Furthermore, induction of an immune response in either wild-type or P-selectin-deficient mice did not alter the subset distribution in the two compartments. Therefore, P-selectin deficiency does not alter the morphology of the lymphocyte subset distribution. Data are presented as mean ± standard deviation, and is based on three mice per group.

ient mice resulted in a similar response in recipients compared with using non-bone marrow transplanted mice as donors ($34.7 \pm 11.7 \text{ cm} \times 10^{-3}$ [non-bone marrow transplanted] vs. $35.8 \pm 9.6 \text{ cm} \times 10^{-3}$ [wild-type bone marrow transplanted], $p = \text{n.s.}$, t -test). However, adoptive transfer of immunity from wild-type mice reconstituted with P-selectin-deficient bone marrow, led to a decrease of the inflammatory response in recipient mice (Figure 4). Comparing this decrease to the one observed using P-selectin-deficient mice, no difference can be observed ($9.1 \pm 13.4 \text{ cm} \times 10^{-3}$ from P-selectin-deficient donors, compared with $10.7 \pm 10.4 \text{ cm} \times 10^{-3}$ from wild-type donor mice transplanted with P-selectin-deficient bone marrow). Taken together this indicates that platelet, not endothelial, P-selectin contributes to generation of immunity in DNFB-induced contact hypersensitivity.

To further sustain the importance of platelets in the generation of immunity in this model, we induced a thrombocytopenia in donor mice for adoptive transfer experiments. Induction of thrombocytopenia in donor mice lead to a significant reduction of the ear swelling response in recipient mice (Table 2). Induction of thrombocytopenia reduced platelet counts over 20-fold within 2 hours to $10.0 \pm 4.2/\mu\text{l}$ (determined on day 5).

Discussion

We here demonstrate that platelet, but not endothelial, P-selectin expression contributes to the generation of

immunity in a delayed type hypersensitivity reaction. In contrast, neither endothelial nor platelet P-selectin expression is required for the elicitation phase of a delayed type hypersensitivity response. These differences may be explained by the differential expression of adhesion molecules in lymph nodes compared with the skin. While in lymph nodes the P-selectin ligand peripheral lymph node addressin is expressed in abundance in class I-III levels²⁶ it is only found in chronically inflamed skin.²⁷ This may also explain why in thrombocytopenic mice infusion of activated platelets from wild-type, but not P-selectin-deficient mice restored the inflammatory response in mice chronically exposed to an immunogenic hapten.¹⁷

There are several mechanisms, which may explain the observed effect of platelet P-selectin on the generation of immunity: In principal, the observed effect might be due to impaired migration of antigen-presenting cells to the peripheral lymph nodes, failure to correctly present antigens to naive T lymphocytes, or defects in lymphocyte migration to peripheral lymph nodes. As platelets are exclusively present in the vasculature, the later hypothesis seems most likely. Platelet-facilitated lymphocyte extravasation might be due to one of the following effects, or a combination thereof: (i) by interaction with endothelial cells in the lymph nodes with subsequent deposition of platelet derived pro-inflammatory molecules²⁸ as it has been described in carotid arteries of mice prone to atherosclerosis.² (ii) by forming aggregates with leukocytes *in vivo*.²⁹ Platelet-leukocyte aggregate formation has been shown to alter the adhesive capacity of leukocytes in a P-selectin dependent manner; eg, bound platelets increased expression, enhanced avidity of leukocyte ad-

Donor mice		Recipient mice (adoptive transfer)	Δ ear swelling ($\text{cm} \times 10^{-3}$)	$p =$
Donor BMT	Recipient BMT			
+/+	+/+	+/+		-
P-select ^{-/-}	+/+	+/+		< 0.001
			0 10 20 30 40 50	

Figure 4. Primarily platelet, not endothelial, P-selectin is required to generate immunity. Using BMT mice as donors for adoptive transfer experiments into wild-type C57Bl/6 mice, ear swelling responses were evaluated. Lack of platelet P-selectin expression had a similar effect as mice fully deficient in P-selectin expression. Hence, platelet P-selectin expression seems more crucially involved in generating immunity than endothelial P-selectin. Data from at least six mice per group; statistical comparisons performed using t -test.

Table 2. Thrombocytopenia Hinders Generation of Immunity towards DNFB

Treatment of donor mice	Δ ear swelling ($\text{cm} \times 10^{-3}$) in recipient mice
Isotype control antibody	7.6 ± 1.2
Anti-CD42 antibody	1.5 ± 0.6

Induction of thrombocytopenia and adoptive transfer was performed as described in *Materials and Methods*. Adoptive transfer of lymphocytes from thrombocytopenic mice led to a significant decrease of ear swelling in recipient mice with normal platelet counts ($P = 0.005$, t -test, $n = 4$ mice/group).

hesion molecules and increased the secretion of cytokines.^{4,5} (iii) Formation of platelet-leukocyte aggregates has been shown to mediate leukocyte-endothelial interaction in peripheral lymph nodes of mice.⁹ Platelet mediated leukocyte rolling is capable to reconstitute generation of immunity in L-selectin-deficient mice; while infusion of activated platelets into wild-type mice has no effect.¹⁵ To investigate, if P-selectin deficiency influences the subset distribution (CD3-, CD4-, and CD8-lymphocytes, NK cells, neutrophils, and macrophages) in the adoptively transferred cells, we characterized the leukocyte subset distribution in wild-type and P-selectin-deficient mice 5 days after painting with DNFB. Leukocyte subset distribution is similar in both strains of mice, and induction of an immune response toward DNFB did not lead to significant changes in subset distribution in either wild-type, or P-selectin-deficient mice (Table 2). This, however, does not rule out a contribution of platelet P-selectin might affect extravasation of a subset not identified by our morphological analysis. Hence, the actual molecular events by which platelet P-selectin contributes to the generation of immunity remain uncertain.

Despite these open issues, we believe that our findings have important clinical implications. As inhibition of P-selectin is a very promising therapeutic approach for modulation of inflammatory diseases and tumor metastasis,³⁰ compounds with a (partial) P-selectin inhibitory activity have been developed. Three of these compounds, namely the pan-selectin antagonist Bimosiamose (TBC1269), several heparin preparations and recombinant PSGL-1-Ig-fusion protein either have been or are currently being evaluated in clinical trials.³⁰ Few P-selectin antagonists have been evaluated in clinical trials in patients with inflammatory diseases. Among those, the pan- or selective E-selectin³¹ antagonist Bimosiamose (TBC-1269)³² attenuates late asthmatic reactions following allergen challenge in mild asthmatics.³³ Another trial evaluated the effect of intralesional, subcutaneous bimosiamose injection over a treatment period of 14 days in patients with psoriasis (open-label, $n = 5$). Clinically, a weak, but statistically significant effect was observed.³² Use of P-selectin targeting strategies has to take into consideration that, besides to inhibition of leukocyte extravasation to the sites of inflammation, generation of immunity may be also impaired. This assumption is strengthened by the observation of an increased morbidity and mortality of P-selectin-deficient mice after i.p. inoculation with *Streptococcus pneumoniae*.³⁴

In summary, platelet P-selectin's contribution to the generation of immunity may have two relevant clinical observations: (i) the immunosuppressive effects of P-selectin targeting strategies have to be taken into account. More specifically, anti-P-selectin therapies would be expected to be associated with an increased risk of infectious diseases. (ii) Therapeutics targeting P-selectin may also become a valuable approach in clinical settings, where generation of immunity may be detrimental; eg, in organ transplanted patients.

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