Neurokinin-1 Receptor Agonists Are Involved in Mediating Neutrophil Accumulation in the Inflamed, But Not Normal, Cutaneous Microvasculature: An In Vivo Study Using Neurokinin-1 Receptor Knockout Mice

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Neurokinin-1 Receptor Agonists Are Involved in Mediating Neutrophil Accumulation in the Inflamed, But Not Normal, Cutaneous Microvasculature: An In Vivo Study Using Neurokinin-1 Receptor Knockout Mice

Thong Cao,* Erika Pintér,* Sabah Al-Rashed,* Norma Gerard,† J. Robin Hoult,† and Susan D. Brain‡,‡

We have used tachykinin neurokinin-1 receptor (NK1 receptor) knockout mice to learn of the link between NK1 receptors and neutrophil accumulation in normal naive skin, as compared with inflamed skin. Intradermal substance P (300 pmol) induced edema formation in wild-type mice, but not in NK1 knockout mice, as expected. However, in contrast to IL-1β (0.3 pmol), substance P did not induce neutrophil accumulation in wild-type mice. IL-1β-induced neutrophil accumulation was similar in wild-type and knockout mice, but a significant (p < 0.05) contributory effect of added NK1 agonists, which by themselves have no effect on neutrophil accumulation in normal skin, was observed. The results support the concept that NK1 agonists such as substance P cannot act on their own to mediate neutrophil accumulation in naive skin and provide direct evidence that in inflamed skin, under certain circumstances, the NK1 receptor can play a pivotal role in modulating neutrophil accumulation during the ongoing inflammatory process. We investigated responses to two inflammatory stimuli (carrageenin and zymosan). Neutrophil accumulation was significantly attenuated (p < 0.001) in carrageenin- but not zymosan-induced inflammation in NK1 knockout mice. The carrageenin (500 µg)-induced response was inhibited (p < 0.05) by a NK1 receptor antagonist, SR140333 (480 nmol/kg i.v. at −5 min), in the wild-type group. The bradykinin B1 and B2 receptor antagonists (desArg9-1-Leu9-bradykinin and HOE 140) each reduced neutrophil accumulation to carrageenin in wild-type animals (p < 0.05), but did not cause further reduction of the suppressed response of knockout mice. The results provide evidence that kinin receptors participate in NK1 receptor-dependent neutrophil accumulation in inflamed mouse skin. The Journal of Immunology, 2000, 164: 5424–5429.

A neurogenic component has been identified in many types of inflammatory responses and the acute proinflammatory microvascular effects of neuropeptides such as substance P (SP) have been well documented (1, 2). Neurogenic inflammation is defined as the edema formation, increased blood flow, and inflammatory cell involvement observed after stimulation of sensory nerves and release of neuropeptides. In particular, the activation of capsaicin-sensitive nerve fibers (usually C fibers) to release neuropeptides, of which the best known are members of the tachykinin family (e.g., SP) and calcitonin gene-related peptide has been studied. SP is a potent mediator of increased microvascular permeability acting via NK1 receptors most commonly found on postcapillary endothelial cells or via non-receptor-dependent mast cell-dependent mechanisms, whereas calcitonin gene-related peptide (CGRP) is a potent arteriolar vasodilator. These microvascular effects are most commonly observed as edema formation or increased blood flow, and the importance of neurokinin-1 receptors (NK1 receptors) in neurogenic edema formation is demonstrated in a recent study with NK1 receptor (Sv129 + C57BL/6) knockout mice (3).

There is also evidence that SP has effects on the cellular component in inflammation, in particular on neutrophil accumulation, the subject of this study (1). However, our recent experiments in rat skin in vivo, using a thermal injury model, have revealed that although the early edema formation is tachykinin NK1 receptor dependent, the later neutrophil infiltration occurs independently of NK1 receptor activation (4). Furthermore, neither administration of SP nor stimulation of sensory nerves had any direct effect on neutrophil accumulation during a 5-h experimental period (4). By comparison, there is evidence from inflammatory models, in NK1 knockout mice, that NK1 receptor activation can contribute to neutrophil accumulation. Bozic et al. (5) demonstrated in an immune complex-mediated model in the lung that neutrophil accumulation was significantly inhibited in NK1 knockout mice. They suggested that the early NK1-mediated edema provided plasma constituents for Ag-mediated responses (e.g., complement components), thus amplifying the response independently of an effect on neutrophils. More recently, Ahluwalia et al. (6) have shown that IL-1β-induced neutrophil accumulation is significantly reduced in NK1 knockout mice in a murine air pouch model of inflammation. They suggested that either IL-1β can act to directly release an NK1 agonist from sensory nerves or perhaps an intermediate such as a bradykinin B1 agonist may be involved. The proposals are supported by earlier
results obtained through the use of selective NK1 receptor antagonists (7) and through the use of kinin bradykinin 1 receptor (B1 receptor) antagonists (8) in the same air pouch model in normal mice. Thus, although it is generally described in the literature that activation of NK1 receptors contributes to neutrophil accumulation, it remains contentious, and little is known about the mechanisms involved. In this study, we have investigated the role of NK1 receptors in mediating neutrophil accumulation in skin induced by IL-1β, carrageein, and zymosan using wild-type and NK1 receptor knockout mice.

Materials and Methods

Animals

Mice (Sv129 + C57BL/6) strain, either genetically unaltered wild-type (+/+), or with the gene for the NK1 receptor removed, knockout (−/−) were developed at Perlmutter Laboratory, Children’s Hospital (Boston, MA) (5) and bred at King’s College London (London, U.K.). The mice were raised in a climatically controlled environment and allowed food and water ad libitum. Both colonies of mice displayed normal growth and behavioral characteristics, and both male and female mice (25–30 g) were used in experiments. Experiments were conducted according to the U.K. Animals (Scientific Procedures Act) 1986.

Effect of agents on neutrophil accumulation

Studies were conducted on anesthetized animals, using 2% halothane (Rhone-Merieux, Harlow, U.K.) in gas (95% O2, 5% CO2). Body temperature was maintained at 37–37.5°C using a heating pad controlled by a rectal thermistor probe (Harvard Apparatus, Edenbridge, U.K.). The dorsal skin of the animals was shaved and prepared for intradermal (i.d.) injection (up to 4 sites per mouse, each in a randomly allocated balanced site pattern). The NK1 receptor antagonist SR140333 (gift from Sanofi, Toulouse, France, 480 nmol/kg); the B1 receptor antagonist desArg²Leu⁶bradykinin (Bachem, Saffron Walden, U.K., 3 μmol/kg); the B2 receptor antagonist HOE 140 (I-p-[Arg-Hyp]³, I-o-Tic⁵, I-o-Thi⁷, bradykinin, 100 nmol/kg, Peninsula Laboratories, Merseyside, U.K.) or vehicle controls were injected i.d. into the dorsal skin. The mice were left for 30 min (NK1 agonists) or 4 h (carageein) to allow plasma extravasation to occur. The tachykinin NK1 receptor antagonist T-31 (desGlu²Pro⁵SP) (5425The Journal of Immunology) (3). In comparison, in separate experiments, no effect of SP on neutrophil accumulation in wild-type mice was detected.

Preparation of leukocytes from mouse peritoneal cavity for determining myeloperoxidase activity per neutrophil

Mixed leukocytes were harvested from the mouse peritoneal cavity by injecting leukocyte-rich acute peritonitis. For this, 6% oyster glycogen (Sigma, Poole, U.K.) dissolved in 1 ml isotonic saline was injected i.p. Then 16–20 h later, the mice were sacrificed by cervical dislocation, and 5 ml ice-cold modified HBSS (Sigma) free of Ca²⁺ and Mg²⁺ was injected into the peritoneal cavity. After 30 s of massage, the peritoneal fluid was collected with a syringe and centrifuged at 4°C for 10 min at 400 × g. Supernatant was removed, and any contaminating erythrocytes were lysed by brief exposure to hypotonic saline (0.2% sodium chloride). After toxicity was restored, the cells were centrifuged again (4°C, 10 min, 400 × g). Supernatant was discarded, and the pellet was taken up in 2 ml HBSS containing 1.26 mM Ca²⁺ and 0.9 mM Mg²⁺. Solution (0.1 ml) was mixed with 0.9 ml complete HBSS agar (0.3%). A sample (0.1 ml) was stained with 0.1 ml trypan blue (0.4% solution), and the number of white blood cells was determined using hemocytometry. For determination of the number of neutrophils, cell smears were prepared by cytospin. Thus, 2–3 drops of cells, from a sample count of 5 × 10⁶ cells/ml, were placed in a cytospin cup attached to a microscope slide and spun for 1 min at 1300 rpm in a cytospin centrifuge (Shandon-Cytospin 2, Shandon Scientific, Runcorn, Cheshire, U.K.). Slides (BDH, Poole, Dorset, U.K.) were dried at 18°C until dry, and the cells were fixed in acetone (BDH, Merck, Poole, Dorset, U.K.) for 10 min and air dried, followed by 10 min in Mayer’s hematoxylin (BDH). Slides were then washed with tap water and placed in 1% chroomeotrope 2R, 1% phenol, (11). Microscopic examination showed that 70–75% of the leukocytes are polymorphonuclear neutrophil granulocytes. Aliquots of standard preparations were made up (2.5 × 10⁶ cells/ml) for daily use (12).

Effect of agents on edema formation

Animals were prepared for i.d. injection (up to 4 sites per mouse) of drugs as described above. Plasma extravasation was determined by Evans blue leakage method (13). Evans blue (Sigma; 2.5%) was injected i.v. using 50 μl/g body weight, 5 min before i.d. treatments. After 5 min, NK1 receptor agonists or carrageenin A or vehicle controls were injected i.d. into the dorsal skin. Animals were left for 30 min (NK1 agonists) or 4 h (carageein) to allow plasma extravasation to occur. The tachykinin NK1 receptor antagonist T-31 was then injected into the dorsal skin. The mice were left for 30 min, the skin was removed, and 16-mm-diameter skin pieces were punched out and placed in 1 ml formamide (Biometrics, Skybio, Wyboston, Beds, U.K.). Reactions were performed in a 96-well microtiter plate at room temperature. OD readings at 620 nm were taken at 5-min intervals for 30 min with a microplate reader (Anthos HTIII, Labtech International, Saltzburg, Austria). These values were plotted, and a reaction rate (OD/time) was measured from the initial slope of the curve. A calibration curve was then produced, with the rate of reaction plotted against the number of neutrophils in the standard samples. This was used to convert reaction rates to number of neutrophils for the skin sample homogenates.

Measurement of neutrophil accumulation

The samples were thawed, chopped, and homogenized in a phosphate buffer containing 0.5% hexadecyl trimethylammonium bromide (Sigma) detergents. The homogenate was centrifuged at 13,000 × g for 5 min (Micrcrocentaur MSE, Scotlab, Strathclyde, Lanarkshire, U.K.). Supernatants (1 ml) were then frozen at −18°C. A previously validated method for measurement of neutrophils in the rat skin (4, 9, 10) was adapted for mouse skin. Neutrophil accumulation was measured by comparing myeloperoxidase activity in extracts from known amounts of mouse neutrophils and compared with that at skin sites. Myeloperoxidase activity was determined using 0.1 M H2O2 oxidation of 3,3’-tetramethylbenzidine (Biomass, Skybio, Wyboston, Beds, U.K.). Reactions were performed in a 96-well microtiter plate at room temperature. OD readings at 492 nm were taken every 5 min for a sample count of 5 × 10⁶ cells/ml, were placed in a cytospin cup and centrifuged at 4°C for 10 min at 400 × g. Supernatant was removed, and any contaminating erythrocytes were lysed by brief exposure to hypotonic saline (0.2% sodium chloride). After toxicity was restored, the cells were centrifuged again (4°C, 10 min, 400 × g). Supernatant was discarded, and the pellet was taken up in 2 ml HBSS containing 1.26 mM Ca²⁺ and 0.9 mM Mg²⁺. Solution (0.1 ml) was mixed with 0.9 ml complete HBSS agar (0.3%). A sample (0.1 ml) was stained with 0.1 ml trypan blue (0.4% solution), and the number of white blood cells was determined using hemocytometry. For determination of the number of neutrophils, cell smears were prepared by cytospin. Thus, 2–3 drops of cells, from a sample count of 5 × 10⁶ cells/ml, were placed in a cytospin cup attached to a microscope slide and spun for 1 min at 1300 rpm in a cytospin centrifuge (Shandon-Cytospin 2, Shandon Scientific, Runcorn, Cheshire, U.K.). Slides (BDH, Poole, Dorset, U.K.) were dried at 18°C until dry, and the cells were fixed in acetone (BDH, Merck, Poole, Dorset, U.K.) for 10 min and air dried, followed by 10 min in Mayer’s hematoxylin (BDH). Slides were then washed with tap water and placed in 1% chroomeotrope 2R, 1% phenol, (11). Microscopic examination showed that 70–75% of the leukocytes are polymorphonuclear neutrophil granulocytes. Aliquots of standard preparations were made up (2.5 × 10⁶ cells/ml) for daily use (12).

Statistical analysis

All results are expressed as the mean ± SEM. Statistical differences between groups were evaluated by ANOVA followed by Bonferroni’s modified t test. A threshold of p < 0.05 was considered statistically significant.

Results

Effect of SP and IL-1β on neutrophil accumulation in wild-type and NK1 receptor knockout mice

In the first experiments, the effect of intradermal SP on edema formation and neutrophil accumulation is shown. SP induced significant edema formation in the dorsal skin of wild-type mice, but not knockout mice, as expected (Fig. 1A) (3). In comparison, in separate experiments, no effect of SP on neutrophil accumulation was observed in either wild-type or knockout mice (Fig. 1B; see also Fig. 3). The effect of the cytokine IL-1β on neutrophil accumulation is shown in Fig. 2. IL-1β was injected i.d. (0.03–3.0 pmol/site). The dose-response curve was bell shaped. The results were similar in wild-type and knockout mice, and 0.3 pmol/site IL-1β-induced significant leukocyte recruitment (p < 0.001) in each case.

The effect of coadministration of SP and IL-1β is shown in Fig. 3. As before, SP did not produce neutrophil accumulation when injected alone but significantly potentiated the effect of IL-1β (0.3
pmol/site) over 5 h in wild-type, but not in knockout mice (Fig. 3). The effect of the selective NK₁ receptor agonist, septide ([pGlu₆,Pro⁹]SP(6–11), 30 pmol/site) was also investigated. In a manner similar to that of SP, septide had no effect on neutrophil accumulation when given alone: Tyrode (vehicle), 1.93 ± 0.27; septide, 1.97 ± 0.18 (values given as number of neutrophils × 10⁶/site, mean ± SEM, n = 6) but potentiated IL-1β-induced cell accumulation in wild-type animals after 5 h. The results were as follows: Tyrode (vehicle), 0.95 ± 0.13; IL-1β, 1.9 ± 0.19; IL-1β + septide, 2.52 ± 0.4 (* values given as number of neutrophils × 10⁶/site, mean ± SEM, n = 11; *, p < 0.05 compared with IL-1β alone). These results show that either added SP or an alternative NK₁ agonist can potentiate neutrophil accumulation induced by the cytokine IL-1β.

**Neutrophil accumulation in wild-type and NK₁ receptor knockout mice in response to zymosan and carrageenin**

The effect of neutrophil accumulation induced by either carrageenin or zymosan was investigated in wild-type and NK₁ receptor knockout mice. Increasing doses of i.d. zymosan induced neutrophil accumulation in a dose-related manner in both types of mice. The number of accumulated neutrophil cells did not differ significantly between wild-type and knockout animals (Fig. 4A). This indicates that the activated NK₁ receptor does not play a major role in the neutrophil accumulation observed in response to zymosan. By comparison, carrageenin, induced substantial neutrophil accumulation in normal mice with a bell-shaped dose-response curve, and this was markedly attenuated in NK₁ knockout mice (Fig. 4B). This indicates that the NK₁ receptors must have a role in neutrophil accumulation induced by carrageenin, but not that induced by zymosan. In separate experiments, during 4 h, edema formation induced by carrageenin (500 µg) was partially, but significantly, attenuated in NK₁ knockout mice, as shown in Table I. The hypothesis that NK₁ receptors play a role in neutrophil accumulation induced by carrageenin is supported by additional experiments conducted in wild-type mice pretreated with the selective, long-lasting NK₁ receptor antagonist, SR140333 (4, 14). SR140333 (480 nmol/kg i.v. 5 min before) significantly inhibited NK₁ agonist-induced plasma extravasation, results not shown, in keeping with our previous studies in the rat (4). SR140333 attenuated carrageenin (500 µg/site)-induced neutrophil accumulation induced in wild-type mice in paired experiments to a level that was also observed in the same experimental series in knockout mice.

**FIGURE 1.** Effect of SP (300 pmol/site) on plasma extravasation during 1 h, n = 6–9 (A) and on neutrophil accumulation during 5 h, n = 4 (B) in the dorsal skin of wild-type and NK₁ knockout mice. SP was injected i.d., and results with vehicle (Tyrode-treated sites) are also shown. Results are expressed as mean ± SEM. Statistical significance was evaluated by ANOVA followed by Bonferroni posttest. ***, p < 0.01 vs. Tyrode-treated controls.

**FIGURE 2.** Effect of IL-1β on neutrophil accumulation in the dorsal skin of NK₁ receptor knockout and wild-type mice. Mice received IL-1β (0.01–3 pmol/site i.d.), and cell accumulation was measured 5 h later. Results are expressed as the mean ± SEM, n = 8 per group. Statistical significance was evaluated by ANOVA followed by Bonferroni posttest. ***, p < 0.001 vs vehicle-treated controls.

**FIGURE 3.** Potentiation of cytokine-induced neutrophil accumulation by SP. SP (300 pmol/site) was coinjected with IL-1β (0.3 pmol/site) into the mouse skin. Neutrophil accumulation was measured at 5 h. Results are expressed as mean ± SEM, n = 7–9. Statistical significance was evaluated by ANOVA followed by Bonferroni posttest. *, p < 0.05; **, p < 0.01; ***, p < 0.001 vs. Tyrode’s-treated control sites. #, p < 0.05 compared with corresponding treatment in knockout mice.
These results further suggest that an NK₁ agonist is released to play a pivotal role in carrageenin-induced neutrophil accumulation in normal mice.

Effects of bradykinin receptor antagonists on carrageenin-induced neutrophil accumulation.

The possible mechanisms by which the NK₁ agonist was involved in carrageenin-induced inflammation was further investigated by determining the effect of selective bradykinin B₁ and B₂ antagonists. Pretreatment with either B₁, (desArg⁹[Leu⁸]bradykinin, 3 nmol/kg i.v.) (15) or B₂ (HOE 140, 100 nmol/kg i.v.) (16–18) receptor antagonists inhibited the carrageenin induced neutrophil accumulation in wild-type mice after 5 h (Fig. 6, third set of columns). However, these antagonists did not affect the suppressed response of knockout mice (Fig. 6, fourth set of columns). Furthermore, neutrophil accumulation induced by zymosan (20 μg/site i.d.) was not affected by the bradykinin receptor antagonists in wild-type mice (Fig. 6, fifth set of columns), thus demonstrating the different kinin receptor involvement in neutrophil recruitment between the zymosan and carrageenin models.

Discussion

The results demonstrate that SP or related ligands cannot by themselves induce neutrophil accumulation in normal mouse skin, despite potent effects on microvascular permeability and edema formation. However, added SP, or an NK₁ agonist (septide) potentiates IL-1β-induced neutrophil accumulation in vivo in wild-type mice, indicating an ability of SP to augment neutrophil accumulation, if produced at inflamed sites. The finding that carrageenin-induced neutrophil accumulation is strongly attenuated in knockout compared with wild-type mice provides evidence that tachykinins are released to play a proinflammatory role in neutrophil accumulation in a common model of dermal inflammation. This is supported by the finding that the NK₁ antagonist SR140333 attenuated carrageenin-induced neutrophil accumulation in wild-type mice. Taken together, our results indicate that NK₁ receptors play a pivotal role in neutrophil accumulation induced by carrageenin in the mouse and that kinins are involved in the response.

The lack of effect of SP, NK₁ agonists and endogenous tachykinins to induce neutrophil accumulation in normal skin has been previously demonstrated by this group in the rat (4). The present results show that in the naive skin of the mice under study, SP, at doses up to 300 pmol, that induce substantial edema formation, do not alone induce neutrophil accumulation. These results differ from those observed in the normal mouse lung because SP or an NK₁ agonist both induced edema formation and neutrophil accumulation (19). This may indicate a tissue difference in the ability of NK₁ agonists to induce neutrophil accumulation in vivo. Alternatively, there are studies that indicate that SP can induce

Table 1. Effect of carrageenin on edema formation in the dorsal skin, measured 4 h after intradermal injection

<table>
<thead>
<tr>
<th>Tyrode Vehicle (0.1 ml)</th>
<th>Carrageenin (500 μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (n = 6)</td>
<td>3.41 ± 0.31</td>
</tr>
<tr>
<td>NK₁ knockout (n = 6)</td>
<td>3.91 ± 0.32</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SEM, *, p < 0.05, ***, p < 0.001 vs Tyrode, †, p < 0.05 vs wild-type mice.
neutrophil accumulation into mouse skin as a consequence of its ability to activate mast cells, via an NK$_1$ receptor-independent mechanism. It has been shown that SP can cause neutrophil accumulation in mice, secondary to mast cell degranulation and leukotriene B$_4$ release (20). Our recent study in wild-type and knockout mice, suggests that SP cannot activate mast cells via an NK 1 receptor-independent mechanism. It has been shown that SP can cause neutrophil accumulation in vivo, inducing local neutrophil accumulation after injection into a range of specific tissue sites (21–23). Thus, this mechanism appears to be lacking in these mice, possibly due to differences in specific technical protocols or due to the strain of mice used.

The proinflammatory cytokine IL-1$\beta$ is a potent mediator of neutrophil accumulation in vivo, inducing local neutrophil accumulation after injection into a range of specific tissue sites (21–23). In 1989, Osborn et al. (24) reported that IL-1 was able to augment the adhesiveness of the vascular endothelium. This cytokine, along with others such as TNF-$\alpha$, up-regulate ICAM-1 and E-selectin expression (25). Our results, from experiments conducted in normal skin, indicate that IL-1$\beta$ induced significant neutrophil accumulation at 0.3 pmol/site, but this was not observed with higher doses, with similar results in wild-type and knockout mice. This bell-shaped dose-response curve is in keeping with results from other in vivo studies of IL-1$\beta$-induced neutrophil accumulation, e.g., into a s.c. sponge in the mouse (26). The results clearly indicate that neutrophil accumulation in response to IL-1$\beta$ is not primarily modulated by an NK$_1$ receptor-dependent mechanism and suggests that IL-1$\beta$ does not directly release an NK$_1$ agonist in normal mouse skin. By comparison, Ahluwalia et al. (6) showed, using a 6-day murine air-pouch model with NK$_1$ receptor knockout mice, that NK$_1$ receptors play a significant role in the cellular response to IL-1$\beta$. It is interesting that the murine air pouch model involves skin tissues, but the cells alter during its development such that the lining cells are substantially changed to include macrophage and fibroblast-like cells (27). It is possible that in this chronic inflammatory model IL-1$\beta$ can influence neuropeptide release via mediator systems that are not present in normal naive skin. This suggestion is supported by our results, which show that the combined administration of IL-1$\beta$ with either SP or the selective NK$_1$ agonist sepiptide leads to a potentiation of IL-1$\beta$ neutrophil accumulation in wild-type mice. This is clear evidence that an NK$_1$ agonist, if released at an inflammatory site can potentiate neutrophil accumulation.

We then turned our attention to learning more about the release and role of NK$_1$ agonists in cutaneous inflammation. Our aim was to study mechanisms in a system where endogenous tachykinins may be released and contribute to neutrophil accumulation. Zymosan (yeast cell walls) induces well-defined neutrophil accumulation in many species, including the mouse (28). The chemotactic complement factor C5a plays a major role in this model, but other mediators including mast cell amines, but not bradykinin, have been suggested to contribute to these inflammatory responses in vivo (29, 30). Zymosan induced dose-dependent neutrophil accumulation, but results were similar in wild-type and knockout mice, indicating a lack of functional involvement of NK$_1$ agonists. These results were in contrast to those obtained using carrageenin. Carrageenin (sulfated polysaccharide from seaweed) induces inflammation that includes substantial edema formation (31). The complement factors play a minor role (28, 29, 32), but the involvement of neutrophils (32) and the kinin system (28, 33) has been demonstrated. In addition, there is evidence for a tachykinin neurogenic component (34–36). Our results demonstrated a small but significant decrease in edema formation in knockout compared with wild-type mice, but a substantial and highly significant decrease in neutrophil accumulation. The selective NK$_1$ antagonist SR140333 inhibited neutrophil accumulation induced by carrageenin in wild-type mice, which is in keeping with the suggestion that sufficient tachykinins are released to substantially modulate carrageenin-induced neutrophil accumulation. Thus, the results provide direct evidence that endogenous tachykinins can influence neutrophil accumulation in inflamed skin.

The release of endogenous tachykinins in inflamed skin is of interest. Our results discussed above rule out the possibility that IL-1$\beta$ can directly mediate neuropeptide release. However, there is evidence from studies in the mouse air pouch (6) and the mouse pleural cavity (37), that kinins are involved in mediating release of tachykinins from sensory nerves in inflamed tissues. The released tachykinins, probably SP, then act as NK$_1$ agonists. The kinin B$_2$ receptor, for which bradykinin has high affinity, is constitutively and widely expressed in peripheral tissues. By comparison, the kinin B$_1$ receptor is not normally expressed in basal conditions but is induced in situations of stress, shock, and tissue inflammation (38). The endogenous agonist of the B$_1$ receptor is desArg$_9$ bradykinin, which is formed from bradykinin and elevated in inflammatory exudates (38). We investigated the effect of pretreatment with B$_1$ and B$_2$ receptor antagonists in carrageenin-induced cell accumulation. The inhibitory effect of the B$_1$ and B$_2$ receptor antagonists in wild-type animals but not in knockout mice indicates that kinins are key intermediates of the NK$_1$ receptor-dependent component of carrageenin-induced neutrophil accumulation, acting via both the constitutive B$_2$ receptor and the inducible B$_1$ receptor. These combined results obtained from wild-type and NK$_1$ knockout mice adds further evidence to the suggestion that kinins may act via B$_1$ or B$_2$ receptors to release SP from sensory nerves in skin. The lack of effect when inflammation is induced by zymosan indicates some selectivity in this response. Thus, the present results are indicative that these mediator pathways only become operational in skin under certain circumstances. This selectivity may be related to the presence and activity of other mediators at inflammatory sites. The results extend findings from other laboratories where a similar phenomenon has been observed in inflammation studies in the airways (39), in a murine pleurisy model (37) as well as in the murine air pouch (6, 7).
In conclusion, the results in this study demonstrate that NK1 antagonists such as SP cannot act on their own to mediate neutrophil accumulation in naive skin. By comparison, the results provide direct evidence that in inflamed skin, under certain circumstances, the NK1 receptor can play a pivotal role in the potentiation of neutrophil accumulation during the ongoing inflammatory process. Plasma extravasation and neutrophil accumulation were both attenuated in carrageenin-induced (but not zymosan-induced) inflammation in NK1 knockout mice. The carrageenin-induced cellular response was inhibited by a NK1 receptor antagonist, SR140333, in the wild-type group. Furthermore, B1 and B2 receptor antagonists (desArg9-Leu9-bradykinin and HOE 140) also attenuated neutrophil accumulation to carrageenin in wild-type mice but not in knockout mice. The results provide further evidence that B1 and B2 receptors participate in the NK1 receptor-dependent component of the neutrophil cell recruitment induced by carrageenin.

Acknowledgments
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