Modulation of Skin Norepinephrine Turnover by Allergen Sensitization: Impact on Contact Hypersensitivity and T Helper Priming

Georges J.M. Maestroni
Center for Experimental Pathology, Istituto Cantonale di Patologia, Locarno, Switzerland

The information gathered by dendritic cells during the innate immune response is determinant for the type and strength of the adaptive response. We showed that the sympathetic neurotransmitter norepinephrine influences dendritic cell migration and T helper priming via \( \alpha \) - and \( \beta \)-adrenoceptors. Others have shown that Langerhans cells also express mRNA for \( \beta_1 \)-, \( \beta_2 \)-, and \( \alpha_1\alpha \) - adrenoceptors and that catecholamines may inhibit the antigen-presenting capability via \( \beta_2 \)-adrenoceptors. Here we report that oxazolone, which induces a predominant T-helper-1-type contact hypersensitivity response, but not fluorescein isothiocyanate, which induces a prevailing T-helper-2-type response, inhibits the local norepinephrine turnover in the skin of mice during the first 8 h of sensitization. Oxazolone also induced higher expression of the inflammatory cytokines interleukin-1 and interleukin-6 mRNA in the skin. Lack or blockade of these cytokines as well as inhibition of prostaglandin synthesis, however, did not influence the oxazolone effect. Only the nonspecific anti-inflammatory steroid dexamethasone could neutralize the effect of oxazolone. Furthermore, fluorescein isothiocyanate but not oxazolone sensitization in the presence of the specific \( \beta_2 \)-adrenoceptor antagonist ICI 118,551 enhanced the consequent contact hypersensitivity response as well as the production of T helper 1 cytokines in draining lymph nodes; conversely T helper 2 cytokines were not affected. Thus, the extent of T helper 1 priming in the adaptive response to a sensitizing agent seems to depend also on its ability to modulate the local sympathetic nervous activity during the innate immune response.

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Stress events or an inadequate response to them and anxiety have been associated with the worsening of a variety of immune-based diseases including allergic asthma, psoriasis, atopic dermatitis, and autoimmune disorders (Bennett et al., 1993; Michel, 1994; Ligier and Sternberg, 1999; Marshall and Agarwal, 2000). A crucial immunologic feature underlying most immunopathologies is an altered T helper 1 (Th1)/Th2 balance (Del Prete et al., 1999). A crucial immunologic feature underlying most immunopathologies is an altered T helper 1 (Th1)/Th2 balance (Del Prete et al., 1999). In fact, Th1 cells promote cellular immunity, protecting against intracellular infection and cancer, but carry the risk of organ-specific autoimmunity. Th2 cells promote humoral immunity, highly effective against extracellular pathogens, and are involved in tolerance mechanisms and allergic diseases. The initiation and type of adaptive immune responses is controlled by innate immune recognition mediated by dendritic cells (DC), which produce interleukin-12 (IL-12), a prerequisite for both the activation of innate immunity and the development of Th1 responses (Schnare et al., 2001).

In vivo IL-12 production and/or DC function may be influenced by a variety of microenvironmental factors, however. Recently we and others have begun to investigate the neuronal influence on DC. DC play a crucial role in the initiation of adaptive immune responses and in the induction of T cell differentiation into Th effectors (Medzhitov, 2001). Langerhans cells are DC residing within the epidermis and often lie in apposition with epidermal nerves including both sensory and sympathetic fibers (Torii et al., 1997; Botchkarev et al., 1999). We reported that norepinephrine (NE) may affect Langerhans cell migration (Maestroni, 2000) and provided evidence that the extent of Th1 differentiation in the response to an antigen might be influenced by the local sympathetic nervous activity in the early phase of DC stimulation (Maestroni, 2002). Another recent report shows that Langerhans cells express mRNA for the adrenoceptors (AR) \( \alpha_1\alpha \), \( \beta_1 \), and \( \beta_2 \). Epinephrine and NE could inhibit the ability of Langerhans cells to present antigen for elicitation of contact hypersensitivity (CHS) response and this effect was inhibited by use of the \( \beta_2 \)-adrenergic antagonist ICI 118,551 (Seiffert et al., 2002). More recently we found that, in analogy with Langerhans cells, bone marrow-derived DC express \( \beta_1 \)-, \( \beta_2 \)-, \( \alpha_2\alpha \)-, and \( \alpha_2\alpha \)-AR genes. The physiologic activation of \( \beta_2 \)-AR in DC and Langerhans cells results in stimulation of IL-10, which in turn inhibits the chemotactic response to the homeostatic chemokines CCL19 and CCL21 resulting in a reduced antigen-presenting ability (Maestroni and Mazzola, 2003).

To understand the physiologic relevance of these findings, we investigated whether chemical allergens that
induce CHS may influence the local NE turnover and hence its impact on Langerhans cell functions. Activation of antigen-specific Th2 cells and B cells may, in fact, increase NE release in the spleen and bone marrow (Kohm et al., 2000), and cytokine receptors along with receptors for other inflammatory mediators are present on various types of peripheral nerves, including sensory nerves (Neumann et al., 1997), sympathetic nerves, and sympathetic ganglia (Francis, 1988; Rogausch et al., 1997; Bai and Hart, 1998; Marz et al., 1998).

We reasoned that different chemical allergens might induce a different inflammatory response in the skin and hence affect differently the local sympathetic tone. This, in turn, might result in a different sympathetic influence on Langerhans cell function and the consequent immune response. In our experiments we used oxazolone and fluorescein isothiocyanate (FITC) as chemical allergens in BALB/c mice. Oxazolone is known to induce a Th1-predominant CHS response (Webb et al., 1998; Dieli et al., 1999), whereas the response to FITC is Th2-predominant (Tang et al., 1996; Dearman and Kimber, 2000). Here we show that oxazolone but not FITC sensitization is associated with an inhibition of NE turnover in the skin. This allergen-induced modulation of the sympathetic nervous activity during the innate phase of the immune response depends on inflammatory mediators and contributes to the information gathered by Langerhans cells before migration and Th cell priming.

Results and Discussion

Effect of allergen sensitization on the rate of NE release in the skin NE turnover analysis (Brodie et al., 1966; Kohm et al., 2000) estimates dynamic changes in sympathetic nerve activity that cannot be obtained by the determination of tissue NE concentration alone. In fact, the rate of NE release is balanced by the rate of NE synthesis, resulting in constant tissue concentrations in spite of marked fluctuations of the sympathetic activity (Brodie et al., 1966). Therefore, to measure the specific rate of NE release in the skin, we used a pulse-chase technique that measures the rate of disappearance of tissue 3H-NE over time. In addition, if experimental conditions induce a significant change in the rate of 3H-NE release over time, the rate of NE turnover can be calculated. To determine whether skin sensitization with oxazolone or FITC influences the rate of NE release from local sympathetic nerve terminals, mice were painted on the shaved back with oxazolone or FITC at time 0 and then injected intraperitoneally with 20 μCi of 3H-NE either immediately or after 17 h. Control mice were painted with oxazolone or FITC vehicles alone. Skin samples were collected at the site of antigen deposition either at 1, 4, and 8 h or at 18, 21, and 25 h following sensitization for NE turnover analysis. Together with skin samples, also the local draining axillary, brachial, and inguinal lymph nodes were collected for NE turnover analysis. The results obtained show that oxazolone but not FITC sensitization strongly inhibited NE turnover in the treated skin, but only during the first 8 h after immunization (Fig 1a, b; Table I). No effect on lymph node NE turnover was detected at any time points (data not shown). Oxazolone induced also a significant decrease in the total NE content in the skin (Table I). Due to the fine balancing between NE release and NE synthesis that keeps the tissue catecholamine concentration constant in spite of variations in nerve activity (Brodie et al., 1966), the NE decrease might reflect a mechanism involving inhibition of NE synthesis.

Expression of pro-inflammatory cytokines in the skin upon allergen sensitization Another transient phenomenon that typically occurs in the first hours after antigen challenge is the expression of pro-inflammatory cytokines. Different allergens may, in fact, elicit a different inflammatory response and hence also a different expression of pro-inflammatory cytokine genes in the skin (Holliday et al., 1997). We investigated this possibility in our model and found that indeed oxazolone seems to induce a pro-inflammatory cytokine profile different from that induced by FITC. IL-1β and IL-6 mRNA levels were determined by real-time RT-PCR. Figure 2 shows that oxazolone but not FITC induced high expression of IL-1β at 1, 3, and 6 h after application. At 3 and 6 h, oxazolone induced also a strong expression of IL-6, which, in contrast, remained low after FITC treatment. Both IL-1β and IL-6 and/or other inflammatory mediators were reported to inhibit peripheral noradrenergic neurotransmission (Francis, 1988; Rogausch et al., 1999; Xia et al., 1999). Inflammatory mediators induced in the skin might thus mediate the observed oxazolone effect by acting on a specific receptor expressed on nerve terminal endings (Rogausch et al., 1997; Bai and Hart, 1998; Marz et al., 1998).

Mechanism of the oxazolone-induced NE inhibition We first investigated whether IL-1β and/or IL-6 were involved in the observed phenomenon. We used IL-6 gene deficient mice or mice pretreated with the specific IL-1 receptor antagonist to counteract the oxazolone-induced inhibition of NE turnover. No effect was observed either in IL-6 gene knockout mice or in the IL-1 receptor antagonist treated mice (data not shown). In other experiments we also used TNF-α gene knockout mice or mice pretreated with the cyclooxygenase inhibitor indomethacin with equally negative results. Hence we could conclude that IL-1β, IL-6, TNF-α, and prostaglandins were not involved in the oxazolone-mediated inhibition of the local NE turnover. The increased IL-1β and IL-6 mRNA expression might thus be the effect rather than the cause of the reduced NE turnover in the skin. NE has been widely reported, in fact, to exert anti-inflammatory effects including inhibition of IL-1β and IL-6 production (Élenkov and Chrousos, 2002; Maestroni, 2002; Seiffert et al., 2002; Maestroni and Mazzola, 2003).

To challenge the hypothesis that other inflammatory mediators were indeed involved in the oxazolone effect, the potent and nonspecific anti-inflammatory steroid dexamethasone was taken into consideration. We pretreated groups of mice with dexamethasone (10 mg per kg body weight, subcutaneously) before sensitization with oxazolone or vehicle and then we assessed the local NE turnover. Figure 3 shows that dexamethasone pretreatment abolished completely the effect of oxazolone on NE turnover. In fact, the slope of the specific activity regression lines and
the NE turnover rate (inset) were similar in both oxazolone- and vehicle-treated mice, suggesting that dexamethasone-sensitive factors are indeed the mediators of the oxazolone effect on NE turnover. Further studies are clearly needed to identify these factors among the multiple pathways affected by dexamethasone.

Effect of the β2-AR antagonist ICI 118,551 on CHS response and cytokine production As Langerhans cell and DC antigen-presenting properties have been shown to be modulated by catecholamines, the apparent ability of oxazolone to inhibit the local norepinephrine turnover during the innate response might be relevant in determining the adaptive response. We investigated this possibility, using repeated exposure of mice to either oxazolone or FITC in the presence or absence of the selective β2-AR antagonist ICI 118,551. Repeated exposure to allergens is needed to elicit a divergent cytokine secretion pattern (Dearman et al., 1997). Six days later the CHS response as well as the amount of Th1 and Th2 cytokines released by lymph node cells were measured. Table II shows the influence of ICI 118,551 treatment during sensitization with FITC or oxazolone on Th cytokine secretion pattern and the CHS response. The major difference upon repeated exposure to the allergens concerns IL-5 and, especially, IL-4, which were significantly higher in lymph node cells from FITC-sensitized mice confirming the Th2 priming properties of FITC. Interestingly no other cytokine, including IFN-γ, showed any significant difference between FITC- and oxazolone-sensitized mice. The presence of ICI 118,551 during FITC but not oxazolone sensitization, however, induced a significant increase of IFN-γ and IL-2 production as well as of the CHS response. The CHS response was also higher upon oxazolone sensitization in comparison to FITC sensitization. The presence of ICI 118,551 during FITC sensitization abolished this difference.

Table I. The effect of oxazolone or FITC painting on the rate of NE release in the skin 1–8 h after allergen administration

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Tissue NE (ng per g)</th>
<th>p</th>
<th>Slope</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA</td>
<td>21</td>
<td>39.1 ± 8.3</td>
<td>&lt;0.01</td>
<td>–0.008</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>OXA vehicle</td>
<td>42</td>
<td>53.8 ± 14.7</td>
<td></td>
<td>–0.087</td>
<td></td>
</tr>
<tr>
<td>FITC</td>
<td>21</td>
<td>60.9 ± 12.7</td>
<td></td>
<td>–0.061</td>
<td>n.s.</td>
</tr>
<tr>
<td>FITC vehicle</td>
<td>21</td>
<td>52.3 ± 9.1</td>
<td></td>
<td>–0.057</td>
<td></td>
</tr>
<tr>
<td>OXA + dexamethasone</td>
<td>21</td>
<td>54.1 ± 9.6</td>
<td>n.s.</td>
<td>–0.069</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

The experimental design is described in the legend to Fig 1. The NE values are the mean ± the standard deviation. Slope is relative at the line representing the log cpm of 3H-NE per ng of tissue NE.
These results suggest that the physiologic presence of NE released by sympathetic nerve terminals in the skin influences the response to FITC but not to oxazolone. It seems reasonable to conclude that this depends on the ability of oxazolone to inhibit the local NE turnover, probably by its ability to induce inflammatory mediators in the skin. We have shown that NE may act on β2-AR expressed in epidermal Langerhans cells, decreasing their migration capability possibly by stimulating IL-10 production, which in turn inhibited the chemotactic response to the homeostatic chemokines CCL19 and CCL21. These events led to reduced Th1 priming and CHS response (Maestroni and Mazzola, 2003) confirming a previous report (Seiffert et al., 2002). Hence, the ability of ICI 118,551 to increase Th1 priming and the CHS response to FITC, as well as its inefficacy in oxazolone-treated mice, reveals the existence of a physiologic sympathetic modulation of Langerhans cell function. It should also be noted that ICI 118,551 did not apparently affect Th2 cytokines but only increased Th1 priming. The physiologic function of NE might therefore be that of limiting Th1 priming but not of inducing a Th2 shift. This effect contributes in determining the cytokine profile in the response to certain allergens, however. In general, contact sensitizers induce a Th1 response whereas Th2 responses are observed after exposure to respiratory allergens. Thus, the inflammatory response to contact sensitizers seems to include a blockade of the local NE turnover. This, together with the antigenic challenge, enhances the antigen-presenting ability of Langerhans cells, increasing Th1 priming and the consequent CHS response.

Table II. The effect of ICI 118,551 administered during sensitization on Th cytokine secretion pattern and CHS response to FITC and oxazolone

<table>
<thead>
<tr>
<th></th>
<th>TNF-α (pg per mL)</th>
<th>IFN-γ (pg per mL)</th>
<th>IL-5 (pg per mL)</th>
<th>IL-4 (pg per mL)</th>
<th>IL-2 (pg per mL)</th>
<th>CHS (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC</td>
<td>335 ± 66</td>
<td>8701 ± 3484</td>
<td>360 ± 117b</td>
<td>97 ± 36b</td>
<td>81 ± 42</td>
<td>122 ± 38</td>
</tr>
<tr>
<td>FITC + ICI</td>
<td>386 ± 126</td>
<td>19993 ± 5873a</td>
<td>403 ± 186b</td>
<td>118 ± 56a</td>
<td>152 ± 38a</td>
<td>183 ± 27a</td>
</tr>
<tr>
<td>OX</td>
<td>367 ± 65</td>
<td>15609 ± 2199a</td>
<td>215 ± 92</td>
<td>23 ± 9</td>
<td>102 ± 37</td>
<td>197 ± 30</td>
</tr>
<tr>
<td>OX + ICI</td>
<td>382 ± 86</td>
<td>14909 ± 1974a</td>
<td>196 ± 87</td>
<td>18 ± 6</td>
<td>97 ± 35</td>
<td>218 ± 46</td>
</tr>
</tbody>
</table>

Mice were sensitized to FITC or oxazolone (OX) by repeated exposure in the presence or absence of the selective β2-AR antagonist ICI 118,551. Six days later the Th cytokine secretion pattern in lymph node cells and the CHS response were assessed. The figures represent the mean ± SD of three experiments (12 mice per group).

b p < 0.01 vs FITC.
a p < 0.02 vs OX, OX + ICI.
In the presence of a lower or different inflammatory response such as that elicited by respiratory allergens, NE is not inhibited and it may act on β2-AR in Langerhans cells limiting their Th1 priming ability. Of course, NE might act also on skin components other than Langerhans cells, although our previous findings point to Langerhans cells and DC as main effectors of the immune-modulating properties of NE, at least during the innate immune response (Maestroni, 2000, 2002; Maestroni and Mazzola, 2003).

The early, innate response to allergens (antigens) seems thus to involve the local sympathetic nervous system, which may impact on DC priming properties. Our findings reveal a basic physiologic mechanism that contributes to the information gathered by DC in the early phase of their activation. Future studies should be aimed at detailing the mechanisms and the physiopathologic implications of this surprising neuro-immunologic connection.

Materials and Methods

The experiments have been approved by our institute and by the local veterinary authority.

Mice BALB/c inbred mice were purchased from Harlan, Italy. All the mice used in the experiments were female, 2–4 mo old, and were maintained in our animal room under a standard 12 h photoperiod, at 21 ± 1 °C, with food and water ad libitum.

Drugs and reagents NE, dexamethasone, oxazolone, and FITC were purchased from Sigma, St Louis, MO. The selective NE antagonist ICI 118,551 was purchased from Tocris Cookson, UK.

NE turnover analysis The analysis was performed according to a recently published method with some modifications (Kohm et al., 2000). Mice were painted on the shaved back with 150 μL of 3% oxazolone dissolved in a 4:1 (vol/vol) acetone:olive oil mixture or with 150 μL of 1% FITC in 1:1 acetone: dibutylylphthalate. Control mice were injected intraperitoneally with 20 μCi of 3H-NE in 200 μL of saline plus 0.1% ascorbic acid either at the time of or 17 h following allergen administration. Skin and the draining lymph nodes were collected from the mice 1, 4, or 8 h after 3H-NE injection and stored at −80°C until time of analysis. At each sampling time the mice were sacrificed and a piece of skin never exceeding 3 cm² in size was excised from the mid back on the right side, i.e., from the central part of the painted area. The weight of the skin samples ranged from 70 to 120 mg. Skin samples and lymph nodes were homogenized in cold phosphate saline (pH 7.4) using a Polytron tissue homogenizer (Kinematica, Switzerland) and centrifuged. The supernatant was mixed with activated alumina for 30 min at room temperature. The alumina was then washed with water and the alumina-bound NE was eluted with 400 μL 0.4 M perchloric acid. The concentration of NE in the eluate was measured following high performance liquid chromatography separation and electrochemical detection. The recovery of NE from alumina was approximately 70%. Aliquots (175 μL) of alumina eluates were mixed with 5 mL of scintillation cocktail. The specific activity of the 3H-NE (cpm per ng of NE) was calculated as the quotient of 3H-NE present in the tissue and the total tissue NE content. To determine NE turnover rates (rate constant × tissue NE) the specific activity of tissue NE was plotted as a function of time. The decay of specific activity is a first-order function, a straight line with a negative slope, and decay lines were calculated by the method of least squares. The rate constant represents the fraction of the NE pool replaced per unit time (h⁻¹). Rate constants (k) were calculated from the slope of the logarithm of the specific activity versus time relationship (0.434×−slope). Differences between the slopes of the regression lines were tested by Student’s t test, using the pooled standard errors of sample regression.

Real-time RT-PCR To reveal the expression of pro-inflammatory cytokine gene expression in the skin, mice were painted with oxazolone or FITC as described above and skin samples were taken after 1, 3, and 6 h. Control mice were painted with the vehicles alone. Skin samples were immediately frozen in liquid nitrogen and pulverized in a cold porcelain mortar. Total RNA isolated from the skin was reverse transcribed using random examers and the TaqMan Reverse Transcription kit (Perkin-Elmer Applied Biosystems, Foster City, CA). A relative quantification of IL-1β and IL-6 and was done on an ABI PRISM 7700 Sequence Detector (Perkin Elmer) using predeveloped TaqMan reagents according to manufacturer’s instructions. Amplification of 18S rRNA was done for each sample as endogenous control of the amount and quality of total RNA added to each reaction. Thermal cycling conditions were 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of two-step PCR consisting of 15 s at 95 °C and 1 min at 60 °C. All samples were amplified in duplicate. The threshold cycle Ct, which correlates inversely with the target mRNA levels, was measured as the cycle number at which the reported fluorescence emission increased above a threshold level. For each sample, the amount of target mRNA (IL-1β, IL-6) was expressed as an n-fold difference relative to the amount of target mRNA in control, vehicle-painted skin. The formula used to determine this value is 2ΔΔCt, where ΔCt is determined by subtracting the average 18S RNA value from the average target ΔCt value. The calculation of ΔΔCt involves the subtraction of the ΔCt of vehicle skin from the target ΔCt value.

Assay for CHS to FITC Mice were sensitized on the shaved back with FITC or oxazolone in the presence or absence of ICI 118,551 (100 μM). This treatment was repeated three times, every other day during 6 d. Six days after the last treatment, the mice were challenged by applying 20 μL of 0.5% FITC or 1% oxazolone on the dorsal and ventral sides of the right ear. As a control the left ear was painted with an identical amount of vehicle (acetone:dibutylphthalate, 1:1). The CHS response was determined by measuring the degree of ear swelling of the FITC-painted ear compared with that of the vehicle-treated contralateral ear at 24 h after challenge using a digital micrometric (Mitutoyo, Allschwil, Switzerland). The results were expressed as net ear swelling, which was calculated by subtracting the thickness of the vehicle-treated ear from the thickness of the FITC-challenged ear.

Cytokines in lymph node cells Mice were sensitized on the shaved back with FITC or oxazolone in the presence or absence of ICI 118,551 (100 μM). This treatment was repeated three times, every other day during 6 d. Skin-draining, axillary, and inguinal lymph nodes were collected 6 d after the last sensitization treatment and single-cell suspensions were prepared. The cells were then stimulated with plate-bound anti-CD3 antibodies. Ninety-six-well U-bottom plates were precoated with hamster antimouse CD3 (25 μg per mL, Pharmingen, Allschwil, Switzerland) or hamster IgG isotype control for 90 min at 37 °C. Cells were seeded at 5 × 10⁶ cells per 200 μL well, and incubated in RPMI 1640, 10% fetal bovine serum at 37 °C for 48 h. Next, culture supernatants were collected and the concentration of interferon-γ (IFN-γ), IL-4, IL-5, tumor necrosis factor α (TNF-α), and IL-2 was quantitated by flow cytometry (FACScanibur, Becton Dickinson, Allschwil, Switzerland) using a Cytomtric Baud Array kit (BD Pharmingen). Before collecting the lymph nodes, the CHS response was assessed as reported.

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Address correspondence to: Georges J.M. Maestroni, PhD, Head, Center for Experimental Pathology, Istituto Cantonale di Patologia, PO Box, Via in Selva 24, 6601 Locarno 1, Switzerland. Email: georges.maestroni@ti.ch

References


Brodie BB, Costa E, Dlabac A, Neff NH, Smookler HH: Application of steady state measuring real-time RT-PCR analysis. Research in Biomedicine, Bellinzona, Switzerland, for the possibility of performing real-time RT-PCR analysis.

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References


Dearman RJ, Kimber I: Role of CD4+ T-helper 2-type cells in cutaneous inflammatory responses induced by fluorescein isothiocyanate. Immunology 101:442–451, 2000


Dearman RJ, Kimber I: Role of CD4+ T-helper 2-type cells in cutaneous inflammatory responses induced by fluorescein isothiocyanate. Immunology 101:442–451, 2000


Elenkov IJ, Chrousos GP: Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. NY Acad Sci 966:290–303, 2002


