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Sympathetic nervous modulation of the skin innate and adaptive immune response to peptidoglycan but not lipopolysaccharide: Involvement of β-adrenoceptors and relevance in inflammatory diseases

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Abstract

Disorders of the skin immune activity are implicated in the pathogenesis of acquired inflammatory skin disorders. Inflammatory diseases including psoriasis, atopic dermatitis, lichen planus and vitiligo have also been associated with local alterations of adrenergic mechanisms and emotional stress. Here we show that the β-adrenergic receptors antagonist propranolol along with peptidoglycan, but not LPS, combined with intradermal injection of a soluble protein, shifted the recall memory response to the Th1 type. The specific β2-AR antagonist ICI 118,551 did not reproduce this effect suggesting that inhibition of both β1- and β2-AR caused the Th1 polarization. The underlying mechanism included enhanced local expression of IFN-γ, IL-12 and IL-23 as well as of IFN-β and CXCR3 ligands during the innate phase of the response which resulted in an increase of antigen-positive plasmacytoid dendritic cells (pDCs) in the draining lymph node. In particular, modulation of inflammatory cytokines, and IFN-β inducible genes expression appeared to involve also the β1-AR. Plasmacytoid dendritic cells and IL-23 were recently reported to play a central role in the pathogenesis of Th1-sustained inflammatory skin diseases such as psoriasis. Thus, primary β-adrenoceptors signaling defects or altered sympathetic nervous activity together with selected pattern recognition receptors activation might serve as initiation and/or persistence factors for numerous Th1-sustained inflammatory skin diseases.

Keywords: Sympathetic nervous system; Skin; Acquired inflammatory diseases; β-Adrenoceptors; Pattern recognition receptors; Interleukin-23; Plasmacytoid dendritic cells; Autoimmunity

1. Introduction

The skin is the largest organ of the body and plays a central role in host defence. The epidermis is composed of keratinocytes which function both as physical barrier and early warning system. Immune cells of the epidermis include Langerhans cells and intraepithelial lymphocytes. The dermis is composed of connective tissue produced by fibroblasts. Immune cells resident in the dermis include dermal DCs, mast cells and cutaneous lymphocyte antigen-positive memory T cells. Recently, we and others reported that catecholamines may affect skin Langerhans cells and bone marrow-derived dendritic cells (DCs) migration and antigen presenting ability via adreneoceptors (ARs) (Maestroni, 2000, 2002a; Maestroni and Mazzola, 2003). However, these studies primarily investigated contact hypersensitivity responses using contact allergens. No in vivo evidence was available about a possible adrenergic modulation of innate or adaptive responses elicited by pattern recognition receptors (PRRs) that recognize specific microbial components (Takeda et al., 2003). Besides DCs and immune cells, epithelial cells, endothelial cells and fibroblasts express PRRs and are important players in cutaneous infections and inflammatory diseases (Faure

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et al., 2000; Kang et al., 2006; Sandor and Buc, 2005). Most cells that may react to PRR ligands, including Langerhans cells, DCs and epidermal keratinocytes express also β-ARs (Chen et al., 2002; Elenkov et al., 2000; Maestroni, 2006). The importance of PRRs (in particular of TLRs) and β-ARs has been both suggested to play a role in skin inflammation. However, a possible interplay between TLRs and β-ARs has not been investigated. As far as it concerns TLRs the evidence of their important role in skin disorders such as psoriasis and atopic dermatitis is steadily growing (Kang et al., 2006). The TLR-induced responses may be dependent or independent on the adaptor protein MyD88. Current opinion views MyD88 as essential for inflammatory cytokine production via all TLRs, whereas TRIF is involved in TLR3- and TLR4-mediated MyD88-independent induction of IFN-β (Takeda et al., 2003). In particular, the TLR2 pathway activated by PGN (Dziarski and Gupta, 2005b) is MyD88-dependent and should lead to NF-κB activation and production of inflammatory cytokines only, while that of TLR4 that sense LPS, should lead to the production of inflammatory cytokines and IFN-β (Takeda, 2005).

For the β-adrenergic system, a dysregulation of catecholamine biosynthesis with epidermal norepinephrine levels associated with high numbers of β2-ARs in differentiating keratinocytes has been reported in vitiligo (Schallreuter, 1997). In atopic eczema, a point mutation in the β2-AR gene could alter the structure and function of the receptor, thereby leading to a low density of receptors on both keratinocytes and peripheral blood lymphocytes (Schallreuter, 1997). In psoriasis, β-ARs are downregulated and, interestingly, β-AR blockers may cause psoriasiform, lichen planus-like and eczematous "chronic" rashes (Halevy and Livni, 1993; Hodl, 1985; Steinkraus et al., 1993). Last but not least, the onset and course of dermatologic disorders may be significantly influenced by stress, emotional disturbances, or psychiatric disorders (Chuh et al., 2006).

Here we present evidence that β-ARs may affect the skin response to peptidoglycan (PGN) from *Staphylococcus aureus* but not to LPS from *Escherichia coli*. β-ARs inhibition and PGN injection before primary immunization with ovalbumin (OVA) dissolved in saline resulted in a Th1 shift of the recall memory response. This effect evidently depended on enhanced local expression of inflammatory cytokines as well as of IFN-β and CXCR3 ligands which were associated with an increased number of pDCs in the draining lymph node. Both β1- and β2-ARs inhibition were apparently involved in the observed effects.

2. Methods

2.1. Mice

BALB/c (H-2^d) inbred mice were purchased from Harlan, Italy and transgenic DO11.10 (H-2^d) mice, expressing on 90% of CD4+ T cells a T cell receptor specific for the OVA peptide 323–339, were a generous gift of Dr. Martin-Fontecha, Institute of Research in Biomedicine, Bellinzona, Switzerland. All the mice used in the experiments were female, 2–4 months old and were maintained in our animal room under a standard 12 h photoperiod, at 21 ± 1 °C, with food and water ad libitum. All experiments were authorized by the local veterinary authority.

2.2. Real time RT-PCR

We measured the mRNA coding for TNF-α, IL-1α, IL-7, IL-12, IL-18, IL-23, IFN-γ, IL-10 and the chemokines CCL1, CCL2, CCL5, CCL27. Groups of mice were injected intradermally (i.d.) with PGN or LPS ± the non-selective β-ARs antagonist propranolol (PRO). Total RNA isolated from skin samples was reverse transcribed using random examers and the TaqMan Reverse Transcription kit (Perkin-Elmer Applied Biosystems, Foster City, CA). A relative quantification of cytokine mRNA was done in a Rotor Gene 2000 (Corbett Research) using pre-developed reagents (Applied Biosystems, Foster City, CA, US). 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 according to the manufacturer instructions. All samples were amplified in duplicate. 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. The amount of inflammatory cytokine or IFN-β and CXCR3 ligands mRNA upon PGN or LPS injection was expressed as a *n*-fold difference relative to the amount of mRNA in controls injected with saline only. The formula used to determine this value is $2^{-\Delta\Delta Ct}$, where ΔCt is determined by subtracting the average 18S rRNA value from the average target Ct value. The calculation of ΔΔCt involves the subtraction of the ΔCt of control from the target ΔCt value.

2.3. Adoptive transfer and immunization

Brachial, axillary, inguinal and popliteal lymph nodes were collected from DO11.10 mice. The lymph nodes were teased and the cells were filtered through a 40 μm cell strainer (Falcon) to prepare a single-cell suspensions in RPMI 1640 with 5% FCS. Lymph node cell suspensions from DO11.10 mice contained 40 ± 1.5% of cells expressing the OVA specific T cell receptor as assessed by flow cytometry using PE-conjugated anti-KJ1-26 mAb (Caltag Laboratories Invitrogen, Basel, Switzerland). The cells were then centrifuged, resuspended in PBS and 7.5×10^6 lymph node cells, containing 3×10^6 transgenic KJ1-26+ cells, were injected i.v. into syngeneic BALB/c mice. Forty-eight hours later the mice were then injected intradermally (i.d.) in the shaved back with 100 μl of PBS containing 50 μg of *S. aureus* peptidoglycan (PGN, Sigma, Co., St. Louis, USA) or 2 μg of *E. coli* lipopolysaccharide (LPS, 0127:B8, Sigma) in presence or absence of propranolol (5 μg, Sigma) or ICI 118,551 (5 μg, Tocris Cookson Ltd., UK). After 3 h the mice were injected i.d. in the same site of the back with 100 μl PBS containing 50 μg of ovalbumin (OVA, Sigma). Ten days later the mice were injected sc with 50 μg of OVA dissolved in 25 μl of PBS and emulsified in 25 μl of FCA. After 7 days the mice were challenged again sc with OVA to assess the DTH response and cytokine production in lymph node cell suspensions.

2.4. Cytokines in lymph node cells and delayed hypersensitivity (DTH) response

Groups of mice were immunized as reported above and 17 days later (recall response) challenged with 50 μg OVA in 50 μl PBS in the right hind footpad. The DTH response was assessed by measuring the degree of foot swelling of the OVA-injected foot compared with that of the vehicle-treated contralateral foot at 48 h after challenge using a digital micrometer (Mitutoyo, Japan). To assess cytokine production, the mice were sacrificed 17 days after immunization and the draining inguinal lymph node cells were obtained, seeded in microplates at 2×10^5 cells/200 μl/well, and incubated in RPMI 1640, 10% FCS at 37 °C for 48 h in presence of 0, 10 and