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Epicutaneous immunization with hapten-conjugated protein antigen alleviates contact sensitivity mediated by three different types of effector cells

Monika Majewska-Szczepanik[1], Anna Strzępa[1], Iwona Drożyńska[1], Sylwia Motyl[2], Tomasz Banach[3], Marian Szczepanik[1]

[1]Department of Medical Biology, Jagiellonian University College of Medicine, Kopernika 7, PL 31-034 Kraków, Poland

[2]Department of Orthodontics, Jagiellonian University College of Medicine, Montelupich 4, PL 31-155 Kraków, Poland

[3]Department of Cardiology and Internal Medicine, L. Rydygier Specialistic Hospital, os. Złotej Jesieni 1, PL 31-826 Kraków, Poland

Correspondence: Marian Szczepanik, e-mail: mmszczep@cyf-kr.edu.pl

Abstract:
Background: Allergic contact dermatitis (ACD) is a common clinical condition in industrialized countries and often causes occupational diseases. Animal model of contact sensitivity (CS) is commonly used to study ACD in mice and can be induced by skin application of haptens. It has been previously shown that CS is mediated by $CD4^+$ or $CD8^+$ T effector cells. More recently it was found that also liver NK cells can play a role of CS effector cells in mice.
Methods: The aim of the present study was to test whether skin-induced suppression could inhibit CS response *in vivo*.
Results: Here we show that EC immunization of normal mice with hapten conjugated protein antigen prior to hapten sensitization suppresses Th1, Tc1 and NK mediated CS responses.
Conclusions: These data strongly suggest that maneuver of EC immunization may have important implications for designing therapeutic schemes aimed at modulating unwanted immune responses in contact hypersensitivity.

Key words:
contact sensitivity, skin-induced tolerance, suppression, NK cells, hapten

Abbreviations: ACD – allergic contact dermatitis, Ag – antigen, APC – antigen presenting cell, CS – contact sensitivity, DNFB – dinitroflurobenzene, EC – epicutaneous, FCS – fetal calf serum, IFN-γ – interferon γ, Ig – immunoglobulins, LMNC – liver mononuclear cell, NK – natural killer, OVA – ovalbumin, Tc – cytotoxic T cell, Teff – effector T cell, TGF-β – transforming growth factor β, Th1 – T helper 1, Th2 – T helper 2, TNP-Cl – picryl chloride, Ts – T suppressor cell

Introduction

The steadily growing number of persons suffering from allergies including contact sensitivity (CS) to haptens, creates ever-increasing social and economic problems affecting life quality and work capacity. The typical CS response in humans is allergic contact der-

matitis (ACD). It is notable that allergic contact dermatitis resulting from exposure to chemicals in the workplace, constitutes about 30% of all occupational diseases [5, 14]. This type of response may occur due to long-term exposure of the skin to low molecular weight substances, including heavy metals (e.g., chromium, nickel, cobalt), latex, turpentine, fragrances and preservatives in cosmetics, epoxy resins and their hardeners, as well as some drugs applied in ointments (e.g., neomycin).

Topical corticosteroids are the mainstay of treatment, while a variety of symptomatic treatments can provide short-term relief of ACD. However, the definitive treatment of ACD is the identification and removal of any potential causal agents; otherwise, the patient is at increased risk for chronic or recurrent dermatitis. Therefore, there is huge need to develop new therapies, non-invasive and free of side effects, specifically eliminating the undesired inflammatory reaction accompanying ACD.

Studies on animals are a valuable source of information on the role of the immune system in the ACD pathogenesis. The progress in knowledge concerning the functioning of the immune system, and the possibility of manipulating its components, as well as learning about the characteristics of the immune constituents in skin obtained by modern research methods, allows for a better understanding of the nature of immune response in ACD. Great hopes are pinned on chances that the acquired knowledge will facilitate development of effective treatment for ACD.

In experimental animals, including mice, CS response is induced by applying the hapten solution in an organic solvent on previously shaved skin [3]. The CS response is a complex process and many cells are involved along the way, including antigen presenting cells (APC), endothelial cells, mast cells, antigen-specific B1 cells, NKT lymphocytes, two types of effector T cells (Teff): $CD4^+$ Th1 or $CD8^+$ Tc1 and peripheral blood leukocytes (monocytes and neutrophils) [2, 7]. It is worth to mention the studies conducted in numerous research centers in recent years suggesting that not only $CD4^+$ Th1 and $CD8^+$ Tc1 lymphocytes which release IFN-γ, but also that $CD8^+$ $T_{IL\text{-}17}$ IL-17-producing lymphocytes play a significant role in CS response [20, 25]. Finally, the discovery by von Adrian et al. proving that NK cells may act as effector cells in CS in mice, was a breakthrough in research on the mechanisms involved in CS response [16]. Our joint studies demonstrated that the NK cells able to adoptively transfer CS response belong to CXCR6-expressing subset [17].

Materials and Methods

Reagents

Dinitroflurobenzene (DNFB) was obtained from Sigma (St. Louis, MO, USA). 2-Chloro-1,3,5-trinitrobenzene (TNP-Cl, picryl chloride) from Nacalai Tesque (Kyoto, Japan) was recrystallized twice and stored protected from light. RPMI 1640 and fetal calf serum (FCS) were from Life Technologies (Grand Island, NY, USA). Percoll was obtained from GE Healthcare (Piscataway, NJ, USA), whereas anti-DX5 microbeads were purchased from Miltenyi Biotec GmbH (Auburn, CA, USA). DNP-BSA was from Biosearch Technologies, Inc. (Novato, CA, USA), whereas mouse immunoglobulins (Ig) were prepared from CBA/J mouse sera and conjugated with TNP hapten [10, 15]. A single preparation with the level of substitution of 40 TNP (trinitrophenyl) per Ig molecule (TNP_{40}-Ig) was used throughout.

Mice

Specific pathogen-free (SPF) female BALB/c ($H\text{-}2^d$) and CBA/J ($H\text{-}2^k$) mice from the breeding unit of the Department of Medical Biology, Jagiellonian University College of Medicine were used. Mice were rested for at least 1 week before use, maintained under SPF conditions, and used at 6–10 weeks of age in groups of 4–6. All experiments were conducted according to guidelines of Jagiellonian University College of Medicine.

Immunization and elicitation of CS to TNP

Mice were actively sensitized by topical application of 0.15 ml of 5% TNP-Cl in acetone : ethanol mixture (1:3, v/v) to the shaved abdomen and hind feet. Control mice were shaved and painted with acetone-ethanol mixture alone as a sham immunization. Four days later, mice were challenged on both sides of the ears with 10 µl of 0.4% TNP-Cl in olive oil-acetone mixture (1:1, v/v). The subsequent increase in ear thickness was measured 24 h later with an engineer's micrometer (Mitutoyo, Tokyo, Japan) and expressed in units of 10^{-2} mm ± SE [11, 18]. Background increase in ear thickness (~2 units at 24 h), of litter-mate non-immunized animals that were similarly challenged, was subtracted from each experimental group,