International Journal of Pediatric Otorhinolaryngology 88 (2016) 146–152

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: http://www.ijporlonline.com/

Role of group 3 innate lymphoid cells during experimental otitis media in a rat model☆

Chang Gun Cho [a, *], Sung Ho Gong [a], Hee-Bok Kim [a], Jae-Jun Song [b], Joo Hyun Park [a], Yun-Sung Lim [a], Seok-Won Park [a]

[a] Department of Otorhinolaryngology-Head and Neck Surgery, Dongguk University Hospital, Goyang, Republic of Korea
[b] Department of Otorhinolaryngology-Head and Neck Surgery, Korea University College of Medicine, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form
1 July 2016
Accepted 2 July 2016
Available online 6 July 2016

Keywords:
Otitis media
Mucosal immunity
Innate immunity
Haemophilus influenzae
Interleukin-17
Interleukin-22

ABSTRACT

The objective of this study was to evaluate the role of group 3 innate lymphoid cells (ILC3) in the middle ear (ME) mucosal response to bacterial infection in a rat model. To confirm the role of ILC3 in bacterially induced otitis media (OM), the serum concentrations of IL-17 and IL-22 were determined by ELISA, and the tissue expression of IL-17 and IL-22 in infected ME mucosa was assessed by immunohistochemical staining. Immunohistochemical staining of specific cell surface markers was also assessed to confirm the origin of the cells expressing IL-17 and IL-22. Twenty Sprague-Dawley rats were used in the surgically-induced animal model of OM. OM was induced by inoculation of non-typeable *Haemophilus influenzae* into the ME cavity of the rats. The rats were divided into four experimental groups: three infected groups and one control group. Infected groups were subdivided into sets of 5 rats, one for each of the three time points (1, 4 and 7 days post-inoculation). For determination of rat IL-17 and IL-22 levels in infected rats and control rats, infected or control ME mucosa sections were analyzed by immunohistochemistry with specific antibodies directed against IL-17 and IL-22. Immunohistochemical staining for CD3, RORγt, and NKp46 were also conducted on the samples to confirm the origin of cells expressing IL-17 and IL-22. IL-17 and IL-22 serum concentrations were significantly increased in the infected rats compared to control rats. Immunohistochemical staining revealed increased IL-17 and IL-22 expressions in all infected ME mucosae from the first day after inoculation. In addition, the results of tissue staining for the specific surface markers were negative for CD3 and NKp46, but were highly positive for RORγt. IL-17 and IL-22 revealed their association with the bacterially induced proliferative and hyperplastic responses of ME mucosa, which are characteristic features in pathogenesis of OM. Surface marker examination showed that the source cells for IL-17 and IL-22 seemed to be lymphoid tissue inducer (LTi) cells. The results suggest that LTi cells release IL-17 and IL-22, and play a significant role in both the early phase of OM induction and recovery from it.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Responses of the immune system against various microorganisms are broadly classified as innate immunity and the acquired immunity. Unlike the acquired immune system, which features antigen specific receptors, the innate immune system has no antigen specificity or functional immunological memory. Innate lymphoid cells (ILCs) are morphologically similar to lymphoid cells and secrete various cytokines, but do not express antigen receptors like the T-cell receptor [1,2]. ILCs are part of the innate immune system and play a fundamental role in the immune responses to infection, repair of damaged tissues and maintenance of homeostasis [3,4].

Many cells classified as ILCs are known. Of this group, the natural killer (NK) cell is the only cell type that is directly cytotoxic to its target cells. Therefore, ILCs can be classified as cytotoxic ILCs, NK cell, and non-cytotoxic ILCs. NK cells and non-cytotoxic ILCs are further categorized into three subgroups according to their specific transcription factors, a reflection of the cytokines they produce and their specific functions within the cells [5–8].

☆ This article was presented as a poster at the ARO 39th MidWinter Meeting in San deigo, California, USA, February 20–24, 2016, Association for Research in Otolaryngology.

* Corresponding author.
E-mail address: cho69@dumc.or.kr (C.G. Cho).

http://dx.doi.org/10.1016/j.ijporl.2016.07.001
0165-5876/© 2016 Elsevier Ireland Ltd. All rights reserved.

NK cells, a member of group 1 ILCs (ILC1), are cytotoxic like the $CD8^+$ cytolytic T cell and secrete IFNγ. IFNγ-producing, non-cytolytic ILC1 functions in a similar way as the T helper 1 cell (Th1) and exists in the mucosal epithelium. T-bet transcription factor is required for development and function of these cells [8–10].

Group 2 ILCs (ILC2) are involved in initial anti-parasitic immunity and allergic inflammatory response by secreting IL-4, IL-5, IL-6, and IL-13, thereby functioning in a way similar to T helper 2 (Th2) cells. The transcription factor GATA3 is crucial in development and maintenance of ILC2 [11–14].

Lymphoid tissue inducer (LTi) cells, which enhance the formation of lymphoid organs, are included in group 3 ILCs (ILC3). ILC3 under the direction of the transcription factor RORγt produce IL-17 and IL-22 in response to IL-23 and IL-1beta. In this way, ILC3 are involved in the mucosal immune response and maintenance of homeostasis [15–17]. In particular, IL-17 and IL-22 expressed by ILC3 promote inflammatory reactions, increase the expression of anti-bacterial peptides including β-defensin, and promote epithelial hyperplasia. Also, functional abnormalities of IL-17 and IL-22 are associated with many inflammatory autoimmune diseases, such as rheumatic arthritis [18,19], psoriasis [20,21], multiple sclerosis [22,23], and inflammatory bowel disease [24,25]. Recent studies of IL-17 and IL-22 have also led to a better understanding of the mechanism underlying these diseases [26–28].

Otitis media (OM) is common in children and infants. About 50%–70% of children experience an OM before the third year of life. Without proper treatment, complications due to infections, such as mastoiditis, brain abscess, and meningitis, may occur. Also, with a delay in language learning, issues with behavior and education may also occur due to prolonged hearing loss.

Many factors, such as a bacterial infection and dysfunction of the Eustachian tube, may combine to cause OM. The most diagnostic pathologic change of OM is transformation and hyperplasia of the middle ear (ME) mucosa. The ME mucosa is 15–20 μm thick, but irritation including bacterial infection or trauma causes the mucosa to thicken to over 1000 μm, and mucosal epithelium transits from a simple columnar epithelium to a pseudostratified columnar epithelium having cilia and secretory function [29]. These changes are generated through the inflow of various inflammatory cells into the mucosa and are the cause of various symptoms of OM.

The molecular cause-and-effect for the ME hyperplasia in OM is yet to be determined and is an active area of research. Exploration of the pathogenesis of ME mucosal change in animal models of OM has shown that mucosal change starts at the early stages of infection and the inflammatory changes disappear within 7 days and change back to the normal mucosa [30]. A variety of signaling molecules, transcription factors, and inflammatory cells are associated with the active inflammatory process of OM. But the precise mechanism of the genesis and development of OM, the exact mechanism of the healing process, and the interrelated role of the mediators have not been identified.

The IL-17 and IL-22 secreted by ILC3 regulate the inflammatory process by increasing the expression of antibacterial peptides, and may play a role in the occurrence of the inflammatory process of the ME. With this hypothesis, we devised the present study to confirm the role of ILC3 in the inflammatory response of the ME mucosa in animal models and investigating the role of ILC3 in the change of the ME mucosa. We compared the serum levels of IL-17 and IL-22 before and after ME infection and confirmed the expression of these cytokines at the ME mucosa in the rat model of non-typeable *Haemophilus influenzae* (NTHi) strain 3655 induced acute OM. We also performed tests to identify the origin of cells secreting IL-17 and IL-22 by using specific surface antigens of CD3, RORγt and NKp46.

2. Materials & methods

2.1. Animals

Twenty Sprague-Dawley rats (7 weeks old and at 300 g in weight) were used. The breeding room was kept at a temperature of 21–23 °C with 40–60% humidity and a 12-h cycle of light and darkness. On arrival, the rats were kept for one week to adjust to the laboratory environment. Sterile water and food were supplied to the rats to their fill. All care and experiments were performed in concordance with the regulations of the Animal Research Institute of Medical Science, Dongguk University, and the study protocol was approved by the Institutional Review Board (ID no. 2015-04123).

2.2. Subdivision of the groups

The 20 rats were randomly divided into four groups, a control group and three experimental groups. The control group had phosphate buffered saline (PBS) injected into their bilateral bullae, and the experimental group were injected with a suspension of NTHi strain 3655 (details below). The infected group was subdivided into subgroups of five rats each. One rat was sacrificed at day 1, 4, and 7 post-inoculation.

2.3. Bacterial strain

NTHi strain 3655 stored at −80 °C was used to induce OM. The bacteria were streaked onto chocolate II agar (BD cat#221169). The batch was incubated overnight at 37 °C with a supply of CO_2. Two colonies were collected, blended with 25 mL Brain Heart Infusion Broth (Teknova, B9500) and 1 mL of Fildes enrichment (Remel, R45037) and were incubated overnight at 37 °C. The mixture was centrifuged to obtain a pellet. A 1:100 diluted solution was made into a bacterial suspension of 10^5 NTHi cells/mL and was used in the experiment.

2.4. Induction of OM

All rats were first checked for abnormalities such as middle ear effusion by otoscopy. The anesthetic used for the animals was made of 100 mg/mL of ketamine, 100 mg/mL of Xyalzine and 10 mg/mL of Acepromazine. The solution used at 0.1 mL/100 g of rat weight was injected into the rat thigh muscle. Under anesthesia, the rat was held in the supine position. The operation was performed under surgical microscopy guidance. Anterior neck was disinfected with betadine and a 3-cm vertical incision was made. The soft tissue was dissected and both bulla was exposed by pushing aside the strap muscle and the submandibular gland. A 25-gauge needle tip was used to pierce a hole at bulla tip, and 0.05 mL of bacterial suspension was injected into the hole.

2.5. Otomicroscopic findings

A model AM4113TL handheld digital microscope-USB (Dino-Lite, New Taipei City, Taiwan) was used to check the inflammation of the ME mucosa and middle ear effusion. The same anesthetic solution was used at day 1, 3, 5, and 7 post-inoculation to take a picture of the tympanic membrane and to check the progress of OM.

2.6. Serum concentration of IL-17 and IL-22

The rats were anesthetized and 0.5–1 cc of blood was collected from the lateral tail vein at each date. Blood samples were collected in BD Microtainer® SST™ (REF 365967) and centrifuged. The

Download English Version:

https://daneshyari.com/en/article/4111402

Download Persian Version:

https://daneshyari.com/article/4111402

Daneshyari.com