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METEORIN-LIKE is a cytokine associated with barrier tissues and alternatively activated macrophages ☆

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Abstract Cytokines are involved in many functions of the immune system including initiating, amplifying and resolving immune responses. Through bioinformatics analyses of a comprehensive database of gene expression (BIGE: Body Index of Gene Expression) we observed that a small secreted protein encoded by a poorly characterized gene called meteorin-like (*METRNL*), is highly expressed in mucosal tissues, skin and activated macrophages. Further studies indicate that *Metrnl* is produced by Alternatively Activated Macrophages (AAM) and M-CSF cultured bone marrow macrophages (M2-like macrophages). In the skin, *METRNL* is expressed by resting fibroblasts and IFNγ-treated keratinocytes. A screen of human skin-associated diseases showed significant over-expression of *METRNL* in psoriasis, prurigo nodularis, actinic keratosis and atopic dermatitis. *METRNL* is also up-regulated in synovial membranes of human rheumatoid arthritis. Taken together, these results indicate that Metrnl represents a novel cytokine, which is likely involved in both innate and acquired immune responses.
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Abbreviations: AAM, alternatively activated macrophages; BIGE, body index of gene expression; FPLCA, Familial Primary Localized Cutaneous Amyloidosis; AD, atopic dermatitis; METRN, Meteorin; METRNL, Meteorin-like; RA, rheumatoid arthritis.

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1. Introduction

Cytokines are signaling molecules that play key roles in many biological processes including hematopoiesis, embryonic development, and immune responses [1]. They are produced by a variety of cell types, and many exhibit pleiotropic effects under inflammatory or homeostatic conditions [2]. Cytokines are low-molecular weight secreted proteins, usually produced by activated leukocytes, that trigger signal transduction pathways upon binding to their specific receptors. Therefore, cytokines represent key molecular messengers through which cells of the immune system communicate with each other in order to develop and control immune responses. While cytokines are crucial for mounting appropriate immune responses, their deregulation is associated with inflammatory abnormalities and/or autoimmune conditions. Blockade of many cytokines and/or their receptors has led to disease alleviation in rheumatoid arthritis, psoriasis, systematic lupus erythematosus and many other indications [3].

In the present study, we asked whether there are still small secreted proteins with cytokine-like characteristics that remain to be identified. To this end, we queried a comprehensive microarray database of gene expression in the human body, a Body Index of Gene Expression (BIGE), which contains gene expression information on more than 105 human cells and tissues [4,5]. This screen led to the identification of several unknown or poorly characterized genes encoding secreted or transmembrane proteins expressed by various cells of the immune system. We have recently reported two of these molecules; the first one is a transmembrane protein that represents a novel biomarker of activated B cells (TSPAN33) [6]; the second is a secreted protein (Isthmin 1) expressed in the skin, mucosa and by NK, NKT and Th17 cells [7].

Here, we describe that a poorly characterized small secreted protein called meteorin-like (metrnl), represents a novel cytokine likely associated with innate and possibly acquired immunity. We show that *METRNL* is highly expressed in barrier tissues (skin, mucosa) and is strongly induced in alternatively activated macrophages (AAM) (or M2 macrophages) and BMM (also termed as M2-like macrophages) [8,9].

METRNL is also associated with human diseases. We investigated its expression in several skin or inflammatory disorders and found that *METRNL* is strongly over-expressed in psoriasis. Furthermore, it is also over-expressed in human rheumatoid arthritis (RA). Taken together, our results indicate that Metrnl encodes a novel cytokine that likely plays a role in inflammatory responses and may be involved in both innate and acquired immune functions.

2. Materials and methods

2.1. BIGE database

The BIGE database has been described [4,5]. We have used it to identify novel genes associated with several tissues or cells [10,11]. To construct the BIGE database, samples from 105 different tissues and cell types of the human body were analyzed for gene expression using U133 2.0 genearrays (Affymetrix, Santa Clara, CA). The resulting data were normalized as described [4], and probesets corresponding to *METRN* (232269_x_at) or *METRNL* (225955_at) were used to determine the expression of the *METRN or METRNL-like* genes in the human body.

2.2. Mice

BALB/C and C57BL/6 mice were obtained from Charles River (Wilmington, MA). Mice were housed in the animal facility of the University of California, Irvine. All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University Of California, Irvine.

2.3. Macrophages

Bone-marrow was isolated from murine femurs and cultured in DMEM (Corning) supplemented with 10% fetal bovine serum (FBS, Life technologies), 2% penicillin–streptomycin (Mediatech, Manassas, VA) and 50 ng/ml M-CSF (BioLegend, San Diego, CA) or 50 ng/ml GM-CSF (BioLegend). After 3 days, non-adherent cells were removed and fresh medium was added. Bone Marrow Derived Macrophages (BMDM) were used after 7 days in culture. To obtain peritoneal cavity macrophages, peritoneal exudate cells (PEC) were collected by lavage and allowed to adhere for 2 h in DMEM supplemented with 10% FBS, and 2% penicillin–streptomycin. Non-adherent cells were then removed and fresh medium was added. Peritoneal cavity macrophages were stimulated with IL-4 (50 ng/ml, BioLegend) to induce AAMs or IFNγ (50 ng/ml, BioLegend). For some experiments, macrophages were also incubated with IL-13 (50 ng/ml, BioLegend), LPS (100 ng/ml, Sigma-Aldrich, St. Louis, MO) or PGE_2 (10 ng/ml, Sigma-Aldrich).

2.4. *Metrnl* ELISA

Mouse Metrnl ELISA was purchased from R&D systems (Minneapolis, MN), and used according to the manufacturer's protocol.

2.5. Collection of skin samples and cell culture

Skin biopsies were collected after obtaining informed consent and approval by the University of Düsseldorf Institutional Review Board from healthy individuals undergoing plastic surgery (n = 11), or from lesional skin from individuals with common skin diseases (psoriasis vulgaris n = 12, atopic dermatitis n = 12, prurigo nodularis n = 6, actinic keratosis n = 6) as part of their diagnosis. Skin biopsies were immediately snap-frozen in liquid nitrogen and stored at −80 °C.

Primary human cells were isolated and cultured as described [12]. For keratinocytes, keratinocyte medium (GIBCO, Invitrogen, Carlsbad, CA) was supplemented with recombinant epidermal growth factor (EGF) and bovine pituitary extract. For fibroblasts, fibroblast medium Quantum 333 (PAA, Pasching, Austria), and for endothelial cells, endothelial cell medium (EGM) MV (Lonza, Basel, Switzerland). Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats by Ficoll-Paque (GE Healthcare, Pittsburgh, PA) density-gradient centrifugation. Control samples for human

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