IL-10 mediates plasmacytosis-associated immunodeficiency by inhibiting complement-mediated neutrophil migration

Upasana Kulkarni, PhD,[a,b] Christian M. Karsten, PhD,[a] Thomas Kohler, PhD,[c] Sven Hammerschmidt, PhD,[c] Kurt Bommert, PhD,[d] Benjamin Tiburzy, MS,[a] Lingzhang Meng, MS,[a] Lara Thieme, MS,[a] Andreas Recke, MD,[e] Ralf J. Ludwig, MD,[e] Karolin Pollok, MS,[f] Kathrin Kalies, PhD,[g] Bjarne Bogen, MD, PhD,[h] Martin Boettcher, PhD,[b] Thomas Kamradt, MD,[b] Anja E. Hauser, PhD,[f] Christian Langer, MD,[i] Markus Huber-Lang, MD,[j] Fred D. Finkelman, MD,[k,l] Jörg Köhl, MD,[a,l] David M. Wong, PhD,[a] and Rudolf Armin Manz, PhD[a] *Lübeck, Jena, Greifswald, Würzburg, Berlin, and Ulm, Germany, Oslo, Norway, and Cincinnati, Ohio*

Background: Plasmacytosis (ie, an expansion of plasma cell populations to much greater than the homeostatic level) occurs in the context of various immune disorders and plasma cell neoplasia. This condition is often associated with immunodeficiency that causes increased susceptibility to severe infections. Yet a causative link between plasmacytosis and immunodeficiency has not been established.

Objective: Because recent studies have identified plasma cells as a relevant source of the immunosuppressive cytokine IL-10, we sought to investigate the role of IL-10 during conditions of polyclonal and neoplastic plasmacytosis for the regulation of immunity and its effect on inflammation and immunodeficiency.

Methods: We used flow cytometry, IL-10 reporter (Vert-X) and B cell–specific IL-10 knockout mice, migration assays, and antibody-mediated IL-10 receptor blockade to study plasmacytosis-associated IL-10 expression and its effect on inflammation and *Streptococcus pneumoniae* infection in mice. ELISA was used to quantify IL-10 levels in patients with myeloma.

Results: IL-10 production was a common feature of normal and neoplastic plasma cells in mice, and IL-10 levels increased with myeloma progression in patients. IL-10 directly inhibited neutrophil migration toward the anaphylatoxin C5a and suppressed neutrophil-dependent inflammation in a murine model of autoimmune disease. MOPC.315.BM murine myeloma leads to an increased incidence of bacterial infection in the airways, which was reversed after IL-10 receptor blockade.

Conclusion: We provide evidence that plasmacytosis-associated overexpression of IL-10 inhibits neutrophil migration and neutrophil-mediated inflammation but also promotes immunodeficiency. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Plasmacytosis, IL-10, immunodeficiency, neutrophil, inflammation*

Plasma cells produce antibodies crucial for immunoprotection but that can also induce inflammation and unwanted tissue destruction.[1-5] Under physiologic conditions, plasma cell numbers are tightly regulated, not exceeding approximately 0.5% of all nucleated cells in lymphoid tissues.[6] However, plasmacytosis, an increase of plasma cell frequencies to greater than the homeostatic level, can occur during severe infectious diseases[7-9] and various autoimmune disorders[10-13] and in plasma cell neoplasms, such as multiple myeloma (MM).[14-16] These conditions are often associated with multiple immunologic defects and increased susceptibility to severe infections, the leading cause of death in patients with MM[17-19] and an important problem in patients with systemic lupus erythematosus (SLE).[20-22] Although therapy-based immunosuppression contributes to this effect, these diseases are also directly associated with immune deficiency.[19,22-24]

In addition to their well-appreciated role as antibody factories, plasma cells also exhibit immunomodulatory functions. For example, they provide negative feedback by suppressing T-follicular helper cells that are crucial for plasma cell generation in T-dependent immune reactions.[25] Furthermore, IL-17 produced by CD138^{hi} plasma cells/plasmablasts mediates protective immunity against *Trypanosoma cruzi* infection.[26] Moreover, recent studies indicate that CD138^{hi} cells can be a major source of IL-10,[27] which can control T cell–mediated inflammation in patients with experimental autoimmune encephalomyelitis.[28]

From [a]the Institute for Systemic Inflammation Research, [c]the Institute of Experimental Dermatology, and [g]the Institute of Anatomy, University of Lübeck; [b]the Institute of Immunology, University Hospital Jena; [c]the Department Genetics of Microorganisms, Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald; [d]the Comprehensive Cancer Centre Mainfranken, University Hospital Würzburg; [f]Charité–University Medicine Berlin, Department of Rheumatology, and German Arthritis Research Center, Berlin; [h]the Centre for Immune Regulation and KG Jebsen Centre for Influenza Vaccine Research, Institute of Immunology, University of Oslo and Oslo University Hospital; the Departments of [i]Internal Medicine III and [j]Orthopaedic Trauma, Hand, Plastic and Reconstruction Surgery, University of Ulm; [k]the Division of Allergy, Immunology and Rheumatology, Department of Internal Medicine, University of Cincinnati College of Medicine, and the Department of Medicine, Cincinnati Veterans Affairs Medical Center; and [l]the Division of Immunobiology, Cincinnati Children's Hospital Medical Center.

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Corresponding author: Rudolf Armin Manz, PhD, Institute for Systemic Inflammation Research, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail: Rudolf.Manz@uksh.de.

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Abbreviations used
Foxp3: Forkhead box protein 3
GFP: Green fluorescent protein
GMD: Goat anti-mouse IgD serum
GST: Glutathione-S-transferase
IC: Immune complex
MM: Multiple myeloma
SLE: Systemic lupus erythematosus
Treg: Regulatory T

IL-10 production has also been reported for human MM cell lines,[29] and high serum IL-10 levels have been found in patients with SLE.[30] However, increased IL-10 production has not been described as a cause of immunodeficiency in patients with MM or other conditions exhibiting plasmacytosis.

Defects in neutrophil functions contribute to the general immunodeficiency associated with SLE and MM.[22,31] Neutrophils are important innate effector cells[32,33] crucial for protection against bacterial pathogens.[34,35] Consistently, bacterial infections are frequently observed in patients with SLE and myeloma.[17,18,22,23] On the other hand, neutrophils drive the acute and chronic inflammation associated with autoimmune diseases.[36]

The complement fragment C5a, one of the most potent neutrophil chemoattractants, is formed by complement activation in response to immune complexes (ICs).[37] For example, local activation of complement and neutrophil infiltration is necessary for the initiation of epidermolysis bullosa acquisita, an antibody-mediated chronic autoimmune skin-blistering disease.[38,39] Accordingly, C5a receptor signaling in neutrophils drives the inflammatory response in the Arthus reaction, the prototypic local immune reaction mediated by acute IC deposition.[40,41]

Here we demonstrate that plasmacytosis-associated IL-10 limits neutrophil-mediated inflammation. Additionally, we provide evidence that the price for this reduced inflammation is a deficiency in neutrophil function, causing increased susceptibility to severe bacterial infections.

METHODS

Mice

C57BL/6 and BALB/c mice were purchased from Charles River (Bar Harbor, Me). IL-10 reporter (Vert-X), BALB/c forkhead box protein 3 (Foxp3) reporter (Foxp3^{eGFP}), and CD19 Cre/IL-10 flox/flox mice and their littermate controls were bred at the animal facility of the University of Lübeck. Experiments were performed at the animal facilities of the Universities of Lübeck and Greifswald.

Experimental epidermolysis bullosa acquisita and polyclonal plasmacytosis

Epidermolysis bullosa acquisita was induced by means of subcutaneous immunization and scored, as previously described.[42] Autoreactive plasma cells were identified by means of flow cytometry, as previously described.[42] For more information, see the Methods section in this article's Online Repository at www.jacionline.org. Collagen VII (amino acids 757-967) was produced, as recently described.[42] Collagen-specific serum antibodies were quantified by means of ELISA. Plates were coated with 500 ng of collagen VII. After blocking, wells were incubated with a 150-fold dilution of the serum samples for 60 minutes. Detection was performed with biotinylated goat anti-mouse IgG antibody (SouthernBiotech, Birmingham, Ala), followed by streptavidin-coupled alkaline phosphatase (Roche Diagnostics, Mannheim, Germany) and ALP (Roche Diagnostics GmbH). Polyclonal plasmacytosis was induced by means of intraperitoneal injection with 200 μL of goat-anti mouse IgD. Some groups received anti–IL-10 receptor (clone IBI.3, a generous gift from DNAX, Palo Alto, Calif).

Murine myeloma

As previously described,[43] murine myeloma was induced by means of intravenous injections of MOPC315.BM cells (5×10^5 cells) stably transfected with eGFP. MOPC315.BM myeloma-specific anti-DNP IgA antibodies were quantified by means of ELISA. Briefly, plates were coated with 10 μg/mL DNP-BSA/PBS (1 hour at room temperature). Nonspecific binding was blocked with 1 mg/mL BSA/PBS. Subsequently, sera were incubated for 1 hour at room temperature. Detection was done with biotinylated goat anti mouse IgA (SouthernBiotech), followed by streptavidin-coupled alkaline phosphatase (Roche Diagnostics) and ALP (Roche Diagnostics).

C5a-mediated peritoneal inflammation

Mice were injected intraperitoneally with goat anti-mouse IgD serum (GMD) or goat serum (0.2 mL) and 6 days later were injected (intraperitoneally) with anti–IL-10 receptor antibody (0.5 mg) or rat IgG. One day later, mice were injected with C5a (200 nM, 100 μL, administered intraperitoneally). After 4 to 5 hours, mice were killed, and neutrophil numbers in peritoneal lavage fluid were determined by means of flow cytometry.

Infection and bioluminescent optical imaging

BALB/c mice with or without plasmacytoma (MOPC315.BM) were infected intranasally with bioluminescent pneumococci (*Streptococcus pneumoniae* D39lux).[44] For this purpose, pneumococci were cultured to the exponential phase (A_{600} = 0.35) in THY medium supplemented with 10% heat-inactivated FBS (Gibco by Life Technologies, Grand Island, NY) and centrifuged, after which the infection dose was adjusted to 5.0×10^8 colony-forming units in 20 μL. Before intranasal infection, mice were anaesthetized by means of intraperitoneal injection of ketamine (Ketanest S; Pfizer Pharma, Karlsruhe, Germany) and xylazine (Rompun; Provet AG, Lyssach, Germany). The bacterial suspension was administered intranasally. Bioluminescent optical imaging with the IVIS Spectrum Imaging System (Caliper Life Sciences, Hopkinton, Mass) allowed monitoring of pneumococcal dissemination after intranasal infection.[45,46] At prechosen time intervals after infection, mice were imaged for 1 minute to monitor dissemination of pneumococci. A time series of the images was generated, and the bioluminescent intensity was determined by means of quantification of the total photon emission with the LivingImage 4.1 software package (Caliper Life Sciences).

For more information on antibodies, flow cytometry, ELISA, histology, statistics, and study approval, see the Methods section in this article's Online Repository.

RESULTS

Polyclonal and neoplastic plasmacytosis is associated with increased IL-10 production

B lineage cells with a CD138^{hi} plasma cell/plasmablast phenotype can significantly contribute to IL-10 production and thereby control T cell–mediated autoimmune inflammation.[28] Here, we first tested the possibility that plasmacytosis increases production of immunosuppressive IL-10. This cytokine was detectable in sera from 6 of 8 patients with advanced myeloma. In contrast, it was present only at a relatively low level in 1 of 7 healthy control subjects and undetectable in patients exhibiting