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Orf virus IL-10 reduces monocyte, dendritic cell and mast cell recruitment to inflamed skin

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ABSTRACT

Orf virus (ORFV) is a zoonotic parapoxvirus that causes pustular dermatitis of sheep, and occasionally humans. Despite causing sustained infections, ORFV induces only a transient increase in pro-inflammatory signalling and the trafficking of innate immune cells within the skin seems to be impaired. An explanation for this tempered response to ORFV infection may lie in its expression of a homolog of the anti-inflammatory cytokine, interleukin (IL)-10. Using a murine model in which inflammation was induced by bacterial lipopolysaccharide, we examined the effects of the ORFV-IL-10 protein on immune cell trafficking to and from the skin. ORFV-IL-10 limited the recruitment of blood-derived Gr-1^{int}/CD11b^{int} monocytes, CD11c^{+ve}/MHC-II^{+ve} dendritic cells and c-kit^{+ve}/FcεR1^{+ve} mature mast cells into inflamed skin. ORFV-IL-10 also suppressed the activation of CD11c^{+ve}/MHC-II^{+ve} dendritic cells within the skin, reducing their trafficking to the draining lymph node. These findings suggest that expression of IL-10 by ORFV may contribute to the impaired trafficking of innate immune cells within infected skin.

1. Introduction

Inflammation is the skin's natural response to infection, leading to clearance of pathogens and induction of an adaptive immune response (Pasparakis et al., 2014; Richmond and Harris, 2014). Upon infection, resident skin cells produce pro-inflammatory cytokines that direct the recruitment and activation of innate immune cells (Kawamura et al., 2014; Pasparakis et al., 2014). Keratinocytes and resident mast cells recognise bacteria and viruses through the activation of toll-like receptors (TLRs), which induces the release of antimicrobial peptides that directly kill pathogens and cytokines that recruit and/or activate neutrophils, monocytes and dendritic cells (DC) (Metz et al., 2008). Additional mast cells are recruited to the skin in response to the inflammatory stimuli (Metz et al., 2008; Dahlin and Hallgren, 2015). Neutrophils also quickly infiltrate inflamed skin and clear pathogens by phagocytosis or through the production of reactive oxygen species (ROS) (Mócsai, 2013). Inflammatory monocytes also infiltrate the skin, differentiating into macrophages or DC that phagocytose infected or damaged cells (Shi and Pamer, 2011). DC, and to a lesser extent macrophages, then migrate to lymph nodes to present internalized antigen to prime T cells (Banchereau et al., 2000; Sabatté et al., 2007). This pathogen-specific response creates immunological memory that prevents re-infection.

Orf virus (ORFV), the prototype species of the *Parapoxvirus* genus of the *Poxviridae* family, infects mainly sheep and goats but is transmissible to humans (Fleming et al., 2015). Infection establishes in cuts or abrasions to the skin, inducing pustular lesions that resolve within 6–8 weeks. ORFV can repeatedly reinfect its host, despite induction of an anti-viral immune response (Fleming et al., 1997; Haig et al., 2002a). The mechanism underlying this apparent escape from immunity does not appear to involve impaired memory, as a strong delayed-type hypersensitivity reaction is detected to ORFV antigen (Buddle and Pulford, 1984; Haig et al., 2002a). Although, infection with ORFV induces the production of inflammatory and immune mediators, such as interleukin (IL)-1β, IL-2, tumour necrosis factor (TNF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ and IL-8 (CXCL8), the levels of these cytokines decline as infection progresses (Haig et al., 1996a; Anderson et al., 2001). Despite the long-lasting infections and transient increase in pro-inflammatory signalling, trafficking of innate immune cells within ORFV-infected skin seems to be impaired. Although neutrophils and dendritic cells accumulate below the lesion (McKeever et al., 1988; Jenkinson et al., 1990b, 1991), the number of monocytes in infected skin decreases, as do the proliferating lymphocytes in the afferent lymph duct (Yirrell et al., 1994). Mast cell numbers in the skin also

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remain unchanged during the course of infection (Jenkinson et al., 1990a,b). These findings suggest that while the inflammatory and innate immune response is induced by ORFV, it is suppressed as the infection becomes established. A possible explanation for this immune-suppression may lie in the production of several secreted immune-modulators by ORFV, one of which is a homolog of the anti-inflammatory cytokine IL-10 (Fleming et al., 1997).

IL-10 is a pleotropic cytokine with potent anti-inflammatory and immune-regulatory functions (Moore et al., 2001; Mosser and Zhang, 2008). IL-10 directly inhibits pro-inflammatory cytokine production and the activation and maturation of innate immune cells such as monocytes, DC, and mast cells. IL-10 also suppresses antigen presentation thus indirectly inhibiting T cell activation. ORFV-IL-10 is structurally and functionally similar to mammalian IL-10 (Fleming et al., 2000; Wise et al., 2007). In cell-culture based assays, ORFV-IL-10 inhibits the production of pro-inflammatory cytokines from activated monocytes and inhibits dendritic cell maturation (Haig et al., 2002b; Imlach et al., 2002; Lateef et al., 2003; Chan et al., 2006; Wise et al., 2007). In a murine model, ORFV-IL-10 also dampened pro-inflammatory cytokine production and reduced the macrophage infiltrate in skin wounds (Wise et al., 2014). Here, we report that, in a murine model of skin inflammation, ORFV-IL-10 impairs the recruitment of blood-derived monocytes, DC and mast cells to inflamed skin and prevents the activation and trafficking of DC from the skin to the draining lymph node.

2. Experimental procedures

2.1. Antibodies and recombinant proteins

The rat anti-mouse I-A/I-E (phycoerythrin (PE)-conjugated, clone M5/114.15.2, isotype IgG_{2b}), rat anti-mouse CD11b (biotin-conjugated, clone MI/70, isotype IgG_{2b}), rat anti-mouse c-kit (biotin-conjugated, clone 180627, isotype IgG_{2a}), rat anti-mouse FcεR1 (PE-conjugated, clone MAR-1, isotype IgG_{2b}), their respective isotypes, and PerCP-Cy5-conjugated Strepavidin were from BD Pharmingen. The rat anti-mouse Gr-1 (allophycocyanin (APC)-conjugated clone RB6-8C5, isotype IgG_{2b}), rat anti-mouse CD11c (APC-conjugated, clone HL3, isotype IgG_{2b}) and their isotypes were from R&D Systems. The rat anti-mouse CD34 (Alexa-Fluor 647-conjugated, clone RAM34, isotype IgG_{2a}) and its isotype was from eBioscience. Recombinant murine (m) IL-10, IL-4 and stem cell factor (SCF/c-kit ligand) were from R&D systems. The ORFV-IL-10 was purified by affinity chromatography from supernatant of 293 EBNA cells transfected with pAPEX containing the viral IL-10 gene (Wise et al., 2007).

2.2. Extraction of bone marrow cells and generation of eGFP DC and mast cells

Bone marrow cells were extracted from the tibias and femurs of eGFP transgenic C57BL/6 mice (Faulkner et al., 2000) as previously described. To generate DC, freshly extracted cells (2×10^6 cells ml^{-1}) were cultured in DMEM (Sigma–Aldrich, MO, USA) supplemented with 20 ng ml^{-1} GM-CSF, 0.1 mg ml^{-1} penicillin-streptomycin and 0.1 mg ml^{-1} kanomycin (PSK) and 10% foetal bovine serum (FBS) (Lateef et al., 2010). Cells were then incubated at 37 °C with 5% CO_2. On day 3, cells were fed with fresh medium. The phenotype of the DC was analysed by flow cytometry after 5 days. To generate mast cells, freshly extracted bone marrow cells were cultured for 7 days as described for the generation of DC, then were re-seeded at 2×10^5 cells ml^{-1} in DMEM mixed 1:1 with conditioned medium from WEHI-3 cells that constitutively express IL-3 (Lee et al., 1982) and supplemented with PSK, 10% FBS, 10 ng ml^{-1} IL-4 and 100 ng ml^{-1} SCF (Tsuji et al., 1991). Mast cells were fed fresh medium twice a week and their phenotype was analysed by flow cytometry after 1 and 5 weeks.

2.3. Flow cytometric analysis

This was performed using a FACSCalibur (Becton Dickinson). All flow cytometric data were analysed using CellQuest software.

2.4. Murine skin inflammation model

All animals were obtained from the University of Otago Animal Facility and used with institutional ethical approval. Skin inflammation was induced in C57BL/6 mice by intradermal (ID) injection of LPS (*Escherichia coli* 055:B5, Sigma–Aldrich) as previously described (Lateef et al., 2009, 2010). ORFV-IL-10 or mIL-10 were co-administered with LPS or PBS in a 20 μl volume. After 24 h, animals were euthanized and biopsies taken of the injection sites and untreated skin. The biopsies were weighed prior to digestion in collagenase/dispase solution (Roche Diagnostics) for 6 h. The isolated cells were counted using the haemocytometer and stained for cell surface expression of Gr-1, CD11b, CD11c, MHC-II, c-kit, FcεR1 or CD34, with the respective isotype controls, or fixed in 70% ethanol and stained with propidium iodide. Samples were analysed using flow cytometry counts of 10,000 events, and were normalized to haemocytometer counts and weight of individual skin samples.

2.5. Recruitment of bone marrow-derived cells from blood to skin

Bone marrow cells (3×10^7 cells) or cultured mast cells (1×10^5 cells) from eGFP mice were suspended in PBS then administered by intravenous (IV) injection to recipient animals as previously described (Lateef et al., 2009, 2010). Twenty-four hours after the adoptive transfer, mice received ID injection of PBS or LPS with or without ORFV-IL-10 or mIL-10. After a further 24 h, animals were euthanized and biopsies taken of the injection sites and untreated skin. The biopsies were weighed, digested, counted, stained for cell surface expression and analysed by flow cytometry, as described above.

2.6. DC migration from skin to lymph nodes

DC (1×10^5) cultured from eGFP mice were suspended in PBS then co-administered by ID injection to skin with PBS, or LPS with and without ORFV-IL-10 or mIL-10. After 24 h, animals were euthanized and biopsies taken of the injection sites and untreated skin. The biopsies were weighed prior to digestion in collagenase/dispase solution for 6 h. The draining inguinal lymph nodes were also extracted and digested. The total cells from skin and nodes were determined using haemocytometer counts. Cells were also stained for cell surface expression of CD11c and MHC-II, with the respective isotype controls. For skin samples, 10,000 events were counted and normalized to haemocytometer counts and weight. For node samples, 200,000 events were counted and normalized to haemocytometer counts.

2.7. Statistical analyses

Statistical analyses of raw data was applied using analysis of variance (single factor ANOVA) with significant points of difference determined using Tukey's test.