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Characterization of αβ and γδ T cell subsets expressing IL-17A in ruminants and swine

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ABSTRACT

As part of our ongoing program to expand immunological reagents available for research in cattle, we developed a monoclonal antibody (mAb) to bovine interleukin-17A (IL-17A), a multifunctional cytokine centrally involved in regulating innate and adaptive immune responses. Initial comparative studies demonstrated the mAb recognizes a conserved epitope expressed on orthologues of IL-17A in sheep, goats and pigs. Comparative flow cytometric analyses of lymphocyte subsets stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin revealed differences in expression of IL-17A by CD4, CD8, and γδ T cells across ruminants and swine species. Results in cattle showed the largest proportion of IL-17A$^+$ cells were CD4$^+$ followed by γδ and CD8$^+$ T cells. Further analysis revealed the IL-17A$^+$ γδ T cell subset was comprised of WC1.1$^+$, WC1.2$^+$, and WC1$^-$ subsets. Analysis of the IL-17A$^+$ CD8$^+$ T cell subset revealed it was comprised of αβ and γδ T cell subsets. Results in sheep and goats revealed IL-17A is expressed mainly by CD4$^+$ and CD8$^+$ T cells, with little expression by γδ T cells. Analysis of IL-17A$^+$ CD8$^+$ T cells showed the majority were CD8$^+$ αβ in sheep, whereas they were CD8$^+$ γδ in goats. The majority of the sheep and goat IL-17A$^+$ γδ T cells were WC1$^+$. Results obtained in swine showed expression of IL-17A by CD4, CD8, and γδ T cell subsets were similar to results reported in other studies. Comparison of expression of IL-17A with IFN-γ revealed subsets co-expressed IL-17A and IFN-γ in cattle, sheep, and goats. The new mAb expands opportunities for immunology research in ruminants and swine.

1. Introduction

Studies on the immune response in species other than humans and mice remain constrained by limited monoclonal antibody (mAb) reagent availability for research, especially for cattle, sheep, goats, and swine (Entrican and Lunney, 2012; Entrican et al., 2009; Moreau and Meurens, 2017). An objective of our research program is to address this problem by developing mAbs where there is a critical need (Elnaggar et al., 2016; Grandoni et al., 2017; Park et al., 2015, 2016; Seo et al., 2009). One gap in the reagent repertoire for comparative studies is mAbs for the interleukin-17 (IL-17) family, molecules centrally involved in host defense against myriad infections [reviewed in (Veldhoen, 2017; Weaver et al., 2007)] and various autoimmune diseases, chronic inflammatory disorders and neoplasia [reviewed in (Beringer et al., 2016; Iwakura et al., 2008)].

The IL-17 family is comprised of six members: IL-17A (commonly known as IL-17 and also as CTLA-8; cytotoxic T lymphocyte associated antigen 8), IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25) and IL-17F have been identified based on shared homology in amino acid sequence with highly conserved cysteine residues essential for their 3-dimensional shape [reviewed in (Chang and Dong, 2011; Gu et al., 2013)]. The evolution of the IL-17 family predates the evolution of the T and B cell receptors with evidence of related molecules in jawless vertebrates and invertebrates (Han et al., 2015; Huang et al., 2015; Vivier et al., 2016; Weaver and Hatton, 2009). Initial studies with IL-17A, the founding member of the IL-17 family, identified a subset of CD4 T cells, T

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helper 17 (Th17) cells as the source of IL-17; however, subsequent work demonstrated CD8 T (Tc17) cells, γδ T cells, natural killer (NK), NKT cells, innate lymphoid cells (ILCs), monocytes, and macrophages are also sources of IL-17 (Cua and Tato, 2010; Fry et al., 2016; Srenathan et al., 2016). In mice, γδ T cells and ILCs are the major sources of IL-17, modulating the immune responses before secretion by Th17 CD4 T cells (Cua and Tato, 2010; Papotto et al., 2017).

Among veterinary species, knowledge on the biology of IL-17A is still limited. Mensikova et al. have summarized the progress made in the study of IL-17 in species other than humans and mice (Mensikova et al., 2013). As noted, the ability to identify and fully characterize the function of the leukocyte subsets involved in modulating innate and adaptive immunity in ruminants and swine, is constrained by the limited repertoire of reagents available for in depth studies including mAbs to IL-17A. Since this review, various approaches have been taken to identify and use reagents to define T cell subsets expressing IL-17A in cattle, sheep and swine. The main strategies used in these studies were; screening of available anti-human IL-17A mAbs for cross-reactivity (Stepanova et al., 2012; Wattegedera et al., 2017) or the use of polyclonal anti-bovine IL-17A (Fry et al., 2016; Steinbach et al., 2016).

As reported herein, we describe the development and use of a mAb to IL-17A to characterize expression of IL-17A in αβ CD4 and CD8 T cell and γδ T cell subsets in cattle, sheep, goats, and swine. The study includes demonstration of co-expression of IL-17A with IFN-γ on subsets of CD4, CD8 and γδ T cells in each species.

2. Materials and methods

2.1. Animals

Blood was obtained from steers (n = 4), sheep (n = 4), and goats (n = 4) being maintained on other projects. All animals and experiments were maintained according to Washington State University institutional animal care and use committee guidelines. For swine, cryopreserved peripheral blood mononuclear cells (PBMC) from four animals were used in short term mitogen stimulation as fresh blood was not available.

2.2. Antibodies and reagents

All monoclonal antibodies used in this study are listed in Table 1. All cell cultures were conducted in complete Roswell Park Memorial Institute (RPMI) medium supplemented with 10% bovine calf serum (HyClone, USA), 20 mM HEPES buffer, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

2.3. Bovine IL-17A mAb development and validation of cross-reactivity with sheep, goats and swine

BALB/c mice were immunized subcutaneously at 2 weeks intervals (50 μg/dose) with recombinant bovine IL-17A (bovIL-17A; amino acid sequence 22–153; Kingfisher Biotech, USA) mixed with oil-in-water emulsion adjuvant (Sigma Aldrich, USA). When antibody was detected in the serum by enzyme linked immunosorbent assay (ELISA) using ELISA plates coated with recombinant bovIL-17A (100 ng/well), mice were injected with a final intravenous boost of bovIL-17A. Spleenocytes were harvested and a fusion conducted as previously described to generate hybridomas (Hamilton and Davis, 1995). The primary hybridomas were screened by ELISA using recombinant bovIL-17A. Positive cultures were expanded, cloned and assessed for ability to bind intracellular IL-17A using PBMC stimulated for 6 h with phorbol 12-myristate 13-acetate (PMA; 50 ng/ml) and ionomycin (1 μM/ml) (Sigma-Aldrich, USA) in the presence of brefeldin A (BD Biosciences, USA). One mAb clone was selected for further characterization and validation. The isotype of the selected mAb was determined by ELISA. Validation of the specificity of the IL-17A mAb was determined via SDS-PAGE and western blot analysis using the recombinant bovIL-17A as the target antigen and recombinant bovIL-2, bovIL-4 and bovIFN-γ (Kingfisher Biotech, USA) as control negative antigens. Recombinant bovIL-17B, C, D, E and F, were not available at the time the study was conducted to check potential cross-reactivity of the anti-bovine IL-17A mAb. However, blasting of bovine IL-17A sequence against other family members (IL-17B, C, D, E and F) available sequences at the National Center for Biotechnology Information (NCBI), showed low sequence similarity (37%, 40%, 36%, 28% and 60% respectively). Finally, cross-reactivity of the mAb with sheep, goat and swine IL-17A was determined via SDS-PAGE and western blot analysis using recombinant ovIL-17A, capIL-17A and swIL-17A as target antigens, and ovIFN-γ, capIFN-γ and swIFN-γ as control negative antigens. Labeling with an IgG1 isotype control mAb (ColiS69A) was included in all experiments.

2.4. PBMC isolation and culture

PBMC were isolated by density gradient centrifugation using Histopaque (density 1.077 g/mL; Sigma-Aldrich, USA), and cultured in RPMI (2×10^6/ml). The cultured cells were stimulated with PMA (50 ng/ml) and ionomycin (1 μM/ml) for 6 h with the addition of brefeldin A. Non-stimulated cultures were included as negative

Table 1
List of mAbs used in this study, their isotype, specificity, species reactivity and source.

mAb clone	Isotype	Specificity	Species reactivity	Source
IL-17A2A	IgG1	IL-17A	Bovine, ovine, caprine and swine	WSUMAC, USA
RIF60A	IgG1	IFN-γ	Bovine, ovine and caprine	WSUMAC, USA
ILA11A	IgG2a	CD4	Bovine	WSUMAC, USA
GC1A	IgG2a	CD4	Ovine and caprine	WSUMAC, USA
7C2B	IgG2a	CD8	Bovine, ovine and caprine	WSUMAC, USA
CACT80C	IgG1	CD8	Bovine, ovine and caprine	WSUMAC, USA
GB21A	IgG2b	γδ TCR	Bovine and caprine	WSUMAC, USA
GD3.5	IgG1	GD3.5 antigen on ovine γδ T cells	ovine	WSUMAC, USA
ILA116A	IgG3	CD45R0	Bovine, ovine and caprine	WSUMAC, USA
BAQ4A	IgG1	WC1	Bovine, ovine and caprine	WSUMAC, USA
ILA29A	IgG1	WC1	Bovine	WSUMAC, USA
BAQ89A	IgG1	WC1.1	Bovine	WSUMAC, USA
CACTB32A	IgG1	WC1.2	Bovine	WSUMAC, USA
PT90A	IgG2a	Swine CD4	Swine	WSUMAC, USA
PT81B	IgG2b	Swine CD8	Swine	WSUMAC, USA
PGBL22A	IgG1	Swine γδ TCR	Swine	WSUMAC, USA