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Molecular cloning of the Swine IL-4 receptor α and IL-13 receptor 1-chains: effects of experimental *Toxoplasma gondii*, *Ascaris suum* and *Trichuris suis* infections on tissue mRNA levels

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Abstract

IL-4 and IL-13 are multi-functional cytokines with overlapping roles in the host defense against infection. Equally important in the regulation of IL-4 and IL-13 are their associated receptors. Though, their functional receptor complexes and signaling pathways are intricate and in some cases, share common elements, the specificity of the responses, nonetheless, resides in the structure and binding of the α-chain components. This report presents the cloning of the swine receptors IL-4Rα and IL-13Rα1 and the effects of parasite infection on their transcription. Pairwise alignment of predicted amino acid sequences indicates that the swine IL-13Rα1 is 86, 83, and 72% similar to canine, human and mouse sequences, respectively. Amino acid sequence conservation is appreciably lower between the swine IL-4Rα sequence and those from equine (72%), human (66%), and mouse (49%); however, noteworthy similarities were observed in their overall predicted secondary structures predominantly among the swine, equine, and human homologues. Relative levels of receptor mRNA in tissues from swine experimentally infected with the protozoan, *Toxoplasma gondii* (*T. gondii*) or the nematodes *Ascaris suum* (*A. suum*) or *Trichuris suis* (*T. suis*), which are known to induce Th1 or Th2 host responses, respectively, were measured by real-time PCR. Results indicated that within 14 days following infection, overall mRNA levels for IL-4Rα and IL-13Rα1 were elevated in *T. gondii*-infected animals and reduced in *A. suum*-infected animals. Levels of swIL-4Rα and swIL-13Rα1 mRNA in *T. suis*-infected animals varied coincidentally with the course of the infection and the location of the analyzed tissue.

Keywords: IL-4 receptor; IL-13 receptor; Swine; Real-time PCR; Gene expression

Abbreviations: BAL, bronchial alveolar lavage; CLN, colonic lymph node; DAI, days after infection; DRN, signal-to-noise ratio; MLN, mesenteric lymph node; PP, Peyer's patch; PCM, proximal colon mucosa; RACE, rapid amplification of cDNA ends; C.V., coefficient of variation

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1. Introduction

Interleukins 4 and 13 are Th2-derived pleiotrophic cytokines which elicit both anti-inflammatory and pro-inflammatory immune responses. Their overlapping roles in regulating B-cell development and effecting IgE-mediated allergenic responses, as well as their effects on monocytes and epithelial cells have been well documented. Amidst their functional and structural similarities, and their physical links to chromosome 5q31 (human), there exist a number of differences, most notably in the kinetics of mRNA transcription and protein secretion, their dependency on different transcription factors, the nature of production among T-cell subsets, and the inability of IL-13 to activate human or mouse T cells.

While IL-4 and IL-13 have been implicated as important mediators of the asthmatic phenotype, treatment with IL-4 has also been shown to play a role in eliminating nematode infections involving *Heligmosomoides polygyrus* (Urban et al., 1991; Svetić et al., 1993), *Nippostrongylus brasiliensis* (Urban et al., 1995), and *Trichuris muris* (Else et al., 1994) via a type-2 cytokine response. Down-regulation of IL-13 using rIL-18 has also been linked to exacerbation of gastrointestinal nematode infections (Helmby et al., 2001). Conversely, the presence of IL-4 or IL-13 in sufficient amounts to abate IFN-γ production has been shown to enhance protozoan infections dependent upon a Th1-type cytokine response for clearance (Heinzel et al., 1989; Chaves et al., 2001); however, current research points to the capacity to produce IFN-γ rather than the presence of IL-4 as a key factor in host resistance to some protozoan parasites (Lehmann et al., 2000). Still, kinetic data from two distinct bovine models of infection involving *Onchocerca ochengi* and *Mycobacterium tuberculosis,* and the dichotomy of concurrent synthesis of IL-4 and IFN-γ, suggest that polarization of the immune response may be more evolutionary than static, and that the timing of cytokine production or experimental treatment can have as great an impact on polarizing the host responses as its presence or absence (Rhodes and Graham, 2002).

Similarities in overlapping functions of IL-4 and IL-13 have led to investigations of their receptor complexes (IL-4R, IL-13R). In mouse and human, both receptors are multivalent. Generally, IL-4R signal transduction requires a heterodimeric receptor complex consisting of both the high-affinity-binding α-chain, and a low-affinity, *trans*-activating γ-chain; however Lai et al. (1996) and Fujiwara et al. (1997) demonstrated that homodimers of IL-4Rα are capable of generating intracellular biochemical signals. In non-hematopoietic cells, which lack the γ-chain, the IL-13Rα1 has been shown to be a functional accessory component of the IL-4Rα complex (Murata et al., 1998). Numerous gene variants of the IL-4Rα chain have been identified, but to date, their functional associations with variations in signal transduction have been tenuous.

Though originally thought to share the common chain with the IL-4Rα and with portions of IL-2, IL-7, IL-9, and IL-15 receptors, the role of IL-13Rα has been somewhat more elusive. It is now believed that IL-13 mediates its effects on the cell through an intricate receptor system, which can involve one of two cell surface proteins, IL-13Rα1 or IL-13Rα2, as well as the IL-4Rα chain, despite the low affinity of IL-4Rα for IL-13. Alone, neither IL-13Rα1 (low-affinity binding) nor IL-13Rα2 (high-affinity binding) affect signal transduction upon association with IL-13; however, in the cloning of the human IL-13αR, Aman et al. (1996) observed that IL-13Rα1 and IL-4Rα form a high-affinity heterodimer capable of transducing IL-4- or IL-13-dependent proliferative signals. Others have proposed a more elaborate IL-13R signaling pathway consisting of no less than four distinct types of receptor complexes (Obiri et al., 1997). These data suggest that interactions between IL-4 or IL-13 and their corresponding receptors, or in the formation of chimeras involving heterologous receptor molecules, are part of an intricate network for cell activation and regulation.

In 1987, Mosmann et al. discovered that the ability of human and mouse IL-4 to stimulate T cells was species-specific. Bonsch et al. (1995) later demonstrated that the human and mouse IL-4Rγ chains interact with their IL-4 counterparts in a fashion geometrically distinct from one another. Recently, in a murine B cell line transfected with either or both the human IL-4Rα or IL-13Rα1 genes, Andrews et al. (2001) showed that the heterodimeric interaction between the subunit receptors and their ability to respond to IL-13 was species-specific. These data imply that deciphering the roles of the IL-4 and

IL-13 receptors in the regulation of immunity to infection are better conducted in their natural hosts. This is of particular importance in light of the multifunctional role that their corresponding soluble receptors can play in regulating IL-4 and IL-13 activities (Sato et al., 1993; Zhang et al., 1997).

Substantial work has been performed using the mouse model to investigate immunity to parasitic infections and in predicting Th1 and Th2 responses in heterologous systems. Within swine, evidence has been advanced suggesting some unique characteristics within the IL-12 pathway. Solano-Aguilar et al. (2002) found that lymphoblast cells were not discernibly stimulated when treated prophylactically with recombinant swine (sw) IL-12 and that mRNA for the swine receptor subunit, swIL-12Rβ2, was not substantially up-regulated during the early course of a protozoal infection. It was determined that substantially lower numbers of swIL-12Rβ2 were present on immune T-cells relative to the bovine system, suggesting a concomitant or possibly delayed mechanism for invoking an IFN-γ response. Given the potential for anomalies within the IL-12/IL-12Rβ2 pathway in swine, the reciprocal relationship between IL-4/IL-13 levels and the production of IFN-γ, and the interplay between membrane-bound and soluble receptors as agonists and/or antagonists of specific immune responses, it is important to obtain a holistic view of immune regulation by studying not only important cytokines, but their receptors as well. Herein, a characterization of the genes encoding the swIL-4Rα and the swIL-13Rα1 is presented. In addition, changes in tissue mRNA levels of these molecules are assessed by real-time PCR in animals experimentally-infected with the protozoan *Toxoplasma gondii* (*T. gondii*) or the nematodes *Ascaris suum* (*A. suum*) or *Trichuris suis* (*T. suis*) which are capable of eliciting Th1- or Th2-type host responses, respectively.

2. Materials and methods

2.1. Tissue preparation

Whole blood was obtained from pigs at the time of sacrifice. Bronchial alveolar lavage (BAL) was performed immediately after killing. Peripheral blood mononuclear cells (PBMCs) were isolated by Histopaque (Sigma, St. Louis, MO) density gradient centrifugation followed by treatment with ammonium chloride (ACK) lysis solution (Biofluids, Gaithersburg, MD) to eliminate the remaining erythrocytes. The isolated cells were subsequently washed two times in PBS, resuspended in Trizol (Invitrogen, Gaithersburg, MD), and vigorously pipetted until all cells were lysed. Other tissues were excised at designated time intervals and immediately flash frozen in liquid N_2. For cloning receptor cDNAs, PBMCs were first cultured for 48 h in the presence of Con A (5 μg/ml) and swIL-4 (10 μg/ml) prior to RNA isolation.

2.2. Animal infections

Seven-week-old Yorkshire–Polland China pigs were given either 4.5×10^5 oocysts of the feline-derived VEG strain of *T. gondii* (Solano-Aguilar et al., 2001), or a total of 10^5 infective *A. suum* eggs (Dawson et al., 2004a). In the case of *T. suis* infections, cross-bred Yorkshire–Danish Landrace–Duroc pigs of both sexes and approximately 10 weeks of age were each given 5000 embryonated eggs (Burden and Hammet (1976). Pigs were killed at indicated times post-infection, and appropriate tissues were processed for RNA isolation. Sampling periods were determined from historically defined points in the parasite infection processes, which are not necessarily congruent between parasite groups. *T. gondii*-infected animals were sampled at 7 and 14 days after infection (DAI), at which times clinical signs of infection peak and abate, respectively. *Ascaris suum*-infected animals were sampled at 14 and 21 DAI coinciding with early L4 development in the intestine, and expulsion or spontaneous cure of non-infectious worms, respectively (Dawson et al., 2004a). For *T. gondii* and *A. suum* infections, $n = 4$ and $n = 3$, respectively, for both infected and control groups.

Samples from *T. suis*-infected animals were taken only from tissues germane to the infection and at 2 week intervals beginning with week 1, in order that the kinetics of the response might be holistically evaluated throughout the course of exposure to the parasite. The numbers of infected and control animals varied as follows: week 1, proximal colon mucosa (PCM) and colonic lymph node (CLN), $n = 6$ for all control and infected groups; week 3, PCM control $n = 3$, infected $n = 5$; CLN, $n = 6$ for both control and infected groups;

weeks 5 and 7, PCM and CLN $n = 6$ for all control and infected groups; week 9, PCM control, $n = 3$, infected $n = 6$, CLN $n = 6$ and $n = 5$ for control and infected animals, respectively; week 11, PCM and CLN, $n = 10$ for all control and infected groups.

2.3. RNA isolation, cDNA synthesis and cloning receptor cDNAs

RNA was extracted from tissues and cells using Trizol as previously described (Solano-Aguilar et al., 2001). For tissues snap frozen in liquid nitrogen, approximately 30 mg samples were ground to a fine powder by mortar and pestle also in the presence of liquid nitrogen. The resultant powder was resuspended in the guanidinium-based lysis buffer and further homogenized by passing five times through a 21-G syringe needle. RNA samples were DNase-treated (Solano-Aguilar et al., 2001) and assessed for integrity and concentration using the Agilent Bioanalyzer 2100 and RNA 6000 Labchip kit (Agilent Technologies, Palo Alto, CA). cDNAs were synthesized using Superscript II reverse transcriptase (Invitrogen, CA) and oligo dT as described (Solano-Aguilar et al., 2001), and used both for cloning receptor sequences and for real-time PCR.

Given the large size of the orthologous mammalian receptor genes and the potential for PCR artifacts, the derived sequences were generated in segments from multiple PCR amplifications using cDNAs as templates, beginning with internal regions of each receptor using human IL-4Rα forward (5′-TTGTTCACCTTTGGACTGGACAGGGAG) and reverse (5′-CAGGTGGCAGGTAAGGGCTGAGTAGAC) primers, or bovine IL-13Rα1 forward (5′-CCACCTGTGACAAATTTGAGTGTCTCTG) and reverse (5′-TGTCTCAGCGTGGCTGTTAATGACTTCTACTTC) primers. Terminal sequences were obtained by 5′ and 3′ RACE. PCR reactions were performed in 50 μl with Advantage 2 Polymerase (BD Biosciences, CA), as recommended by the manufacturer using 0.25 μM of each primer. All amplified fragments were gel-purified using Quiaquick™ and cloned into pCR2.1 by TA overhangs. For each receptor, a consensus sequence was generated from computer alignment of three sequences and identifying base-pair congruence between three or more clones at any given locus. Predicted amino acid sequences were aligned with previously generated IL-4R sequences from human (CAA36672), murine (AAB59727), and equine (AAL87462), and with IL-13Rα1 sequences from human (AAB37127), murine (O09030), and canine (AF314532) using Clustal W (v 1.60). Secondary structure calculations for swIL-4Rα amino acid sequences were performed according to an improved version of the self-optimized prediction method (SOPMA) using default parameters (Combet et al., 2000).

2.4. Real-time PCR analysis of swIL-4Rα and swIL-13Rα1 mRNAs in swine tissue

cDNA equivalent to 100 ng RNA/well was used for each PCR amplification. All probes and primers for real-time PCR were designed using the Primer Express (Applied Biosystems, Foster City, CA) software package. Probes and primers were obtained from Biosource (Camarillo, CA). The sequences and optimum concentrations are as follows: IL-4Rα, 5′-GCAGCGGCATTGTCTATTCA (sense, 300 nM), 5′-TCGTCTTGGCCGTGACACT (antisense, 900 nM), 5′-6FAM-CGTTTGGCACAACCTGCGCTACATG-TAMRA (probe, 200 nM); IL-13Rα1, 5′-CGAGTCTGCTGTTACTGAGCTACAA (sense, 300 nM), 5′-TCCAGGGAGCCAGGTACACT (antisense, 900 nM), 5′-6FAM-CGTTTGGCACAACCTGCGCTACATG-TAMRA (probe, 200 nM).

PCR was performed using a commercially available kit (Brilliant Core Reagent kit, Stratagene, La Jolla, CA) on an ABI PRISM 7700 Sequence Detector System (Applied Biosystems, Foster City, CA) as described (Dawson et al., 2004a,b). Because considerable variation was found in the levels of numerous housekeeping genes resulting from different infectious agents (data not shown), gene-expression data was normalized to the constant amount of RNA/cDNA amplified. This method has recently been proposed as the most reliable means of standardization for relative quantitation of mRNA expression by real-time PCR, provided an accurate measurement of total RNA (i.e. via Agilent Bioanalyzer) can be made (Bustin, 2002).

2.5. Statistical analysis

All statistical analyses were performed using Statview 5.0 for Macintosh (Abacus Concepts, Berkeley,

CA). Data were analyzed for equality of variance using Fisher's F test. If the variance was heterogeneous, the appropriate transformation of the data was performed. All mRNA-expression levels (Ct values) were evaluated by one-way ANOVA to examine the effect of infection. A $P < 0.05$ was considered statistically significant for all analyses.

3. Results

3.1. Cloning and analysis of swIL-4Rα and swIL-13Rα1 cDNAs

Predicted amino acid sequences for swIL-4Rα and swIL-13Rα1 cDNAs and their alignments with orthologous mammalian sequences are shown in Figs. 1 and 2, and are encoded by cDNAs with open-reading frames of 2493 bp (accession no. AY266143) and 1272 bp (accession no. AY266142), respectively. The swIL-4Rα was generated by PCR in four segments and subsequently assembled into a contiguous clone. Segment 1 spanned an internal Pst I site at bs 2038 and the translation stop site; segment 2 extended from the Pst I site to an internal Bam HI site at bs 1377; segment 3 required the inclusion of a synonymous base change at bs 402 to create an Sst I site, and stretched to the Bam HI site, and segment 4 spanned the translation start site and the newly created Sst I site (data not shown). The substantially higher level of mRNA expression and the shorter sequence length permitted cloning of the swIL-13Rα1 as a single product. Sequences shown in Figs. 1 and 2 are consensus data from three or more clones from each amplified fragment.

Both receptor sequences exhibited the WSXWS motif common to type-1 cytokine receptors. In addition to this motif, Bazan (1990) proposed that certain C residues involved in disulfide bridge formation are conserved within many receptor complexes. A putative disulfide bridge within a C[$X_{9/10}$]CXW was found in both receptors beginning with C41 in swIL-4Rα and C131 in swIL-13Rα1. In addition, a C82-C94 bridge in swIL-4Rα and C170-C182 bridge in swIL-13Rα1, which are spatially conserved with respect to their homologous C[$X_{9/10}$]CXW motifs, were also found within the extracellular domains of both the α- and α1-chain receptors from all analyzed species.

Comparison of the swIL-13Rα1 sequence to the predicted amino acid sequences from canine, human and mouse indicated similarity indices of 86, 83, and 72%, respectively. In contrast, when the swIL-4Rα was aligned with equine (72%), human (66%) and mouse (49%), substantially lower levels of congruence were observed, in particular, with the mouse-derived sequence.

3.2. Receptor mRNA-expression levels

When mRNA levels of both swine receptors were examined in tissues from animals infected with *T. gondii*, the swIL-4Rα was significantly up-regulated in the mesenteric lymph nodes (MLN), (CLN), MLN, and jejunal Peyer's patches (PP), slightly up-regulated in the spleen, and moderately down-regulated in the jejunum and thymus at 7 DAI (Fig. 4). SwIL-13Rα1 was similarly up-regulated in the CLN and MLN at 7 DAI, but contrary to the swIL-4Rα, significant up-regulation was observed in the thymus that continued through 14 DAI.

Within *A. suum*-infected animals, swIL-4Rα mRNA levels were up-regulated 14 DAI in the ileum and slightly down-regulated in the ileocecal PP, MLN, jejunum, and thymus (Fig. 4). This same profile remained relatively consistent at 21 DAI. Expression of the swIL-4Rα mRNA in BAL cells was detected in all control animals but was undetectable after 40 amplification cycles in 3/3 infected animals at 14 DAI and in 2/3 animals at 21 DAI. At 14 DAI, swIL-13Rα1 mRNA was significantly up-regulated in the ileum and down-regulated in the ileocecal PP and jejunum. In addition, at 21 DAI swIL-13Rα1 levels showed significant down-regulation in BAL cells and the expression levels in the ileum and jejunum reverted back to levels indicative of control tissues.

For *T. suis*-infected animals, swIL-4Rα mRNA was significantly down-regulated in the PCM 3 and 5 weeks post-infection (Fig. 4). The swIL-13Rα1 mRNA demonstrated a similar pattern of down-regulation, but only after significant induction occurred as early as 1 week following infection. There was selective up-regulation of the both the swIL-4Rα and swIL-13Rα1 mRNA in the CLN during the 3rd week following infection, and in the case of the swIL-4Rα mRNA, again in the 11th week post-infection.

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SwIL4Rα MGWLCPGLTFSVSCLILVWAAGSGVTCVSPGGVRVLEWPICLSDYVSTSTCEWRMAGPVNCSAEF  65
EqIL4Rα ..C.....LP..........-------.S.K..RLTA.F...I.A.....K.DR.T....QL  58
HuIL4Rα .....S..L.P....V.LQV.S.-------.NMK..QE.T.V...M.I.....K.N..T...T.L  58
MuIL4Rα ..R..TKFLT..G....LLVT..-------.SIK..GE.T.F...IR......FLDSA.D..SQL  58
                                               *
        RLSYQLKFFNTENHTTCVPENRAGSVCVCHMLMESIVIVDTYQLDLWAGEQLLWNSSFRPSQNVK 130
        ......NDEFSD.-L..I....EDE....R...DN..SE.V.E......N........K..RH.. 122
        ..L...V.LLS.A-H..I...NG.AG....L..DDV.SA.N.T......Q....KG..K..EH.. 122
        C.H.R.M..EFSENLI.I.R.S.ST......E.NRP.QS.R..ME...EHRQ..QG..S..G... 123

        PLAPRNLMVHANISHTWLLTWSNPYPSESYLYSELTYLVNISNENDPTDFRIYNVTYLGPTLRFP 195
        .R..Q..T...-..............LKNH.W..........K.D.....K......MD....VT 186
        .R..G..T..T.V.D.L.........PDN...NH...A...WS....A..........E.S..IA 187
        ....D..TL.T.V.DE.....N.L...NNL..KD.ISM....R.DN.AE.IV.....KE.R.S.. 188
                            *
        ANTLKSGAAYSARVKAWAQRYNSTWSEWSPSVKWLNYYEEPLEQRLPLGVSISCVVILIICLSCY 260
        .S....R.T.......R..N...........TT.H....Q..................A...... 251
        .S.....IS.R...R....C..T........T..H.S.R..F..H.L....V..I...AV..L.. 252
        I.I.M..VY.T...RVRS.ILTG........IT.Y.HFQL..I.......T...LC.PLF..F.. 253

        FGIIRIKKEWWDQIPNPAHSPLVAIVIQDSQVSLWGKRSRGQEPAKCPRWKTCLTKLLPCFLEHG 325
        .S..K....................L.........Q......................L.... 316
        VS.TK.............R.R....I...A.G.Q.E............H..N............N 317
        .S.TK...I......T..R......I...A..P..D.QT.S..ST.Y.H.....D.....L.K.R 318

        VDRDEDSSKAARNGPSQGPAKAAWRPVEVSKTILWPE--SISVVRCVELFEAQVENEEEE-DEED 387
        LQKE.....TV....F.S.G.S..HT...NH...R..--I....P....C.....S....-V... 378
        MK....PH...KEM.F..SG.S..C...I...V....--.............P..C....EV..E 380
        .KKKT.FP...PTKSP.S.G..G.C.M...R.V....NV.V.....M.....P.Q.V...E..IV 383

        KGSFCPSPENSG-GSFQEGREGIAARLTESLFLDLLGDESGAFSPQGMGQSCLLPPLENASAPMP 451
        R........S..-SG.......V..........G...A.N..LG-----E.......G--..H.. 435
        .....A...S.R-DD........V.............E.N.G.CQ.D..E......SGST..H.. 444
        .EDLSM......GCG...SQAD.M.....N..S...EA.N.GLGQSALAE..SPL.SGSGQ.SVS 448

        WAEFPRVGSPEASSQGKEQPLNPEPSPQATPTQSLASLAFPELPAVIADNPAYRSFSTFLSQSSD 516
        ..RISSA.PQ..A...E.......SN.L..L...PG....T.A...V..........NS...PRG 500
        .D...SA.PK..PPW......HL....P.S....PDN.TCT.T.L...G........NS....PC 509
        ..CL.MGP.E..TC.VT...SH.G.LS-GS.A..APT..CTQV.L.L..........DCC.PAPN 512

        PGELDSDPELAEALEEVEPSLPAAPQPSEPPPTLQPEPETWEQILRQSVLQRRAAPAPASGPSSS 581
        ........Q...H.GQ.D..I.S........TA..........M.......QG.......A.TG- 564
        .R..GP..L..RH......EM.CV..L...TTVP............RN...HG..A..V.A.T.- 573
        ....APEQQQ.DH...E..PS..D.HS.G..--M..-V.S.....HM....HG..AGSTPA.AG- 573

        GYREFVHAVEQGTQDR-RAAGSGPCGEAGYKAFSSLLAGSASCPGTSGLEPSSGESGYKPFQSLP 645
        .....AQV.K..G----G......S................V...Q..V.A....G..R.YE.PD 625
        ..Q.........GTQASAVV.L..P.............S..VS.EKC.FGA....E......D.I 638
        ..Q...Q..K..AAQDPGVP.VR.S.DP.........SSNGIRGD.AAAGTDD.HG......NPV 638

        PGCPE--TPVPTPLFTFGLDMEPPPSPQNPPFPGSSAECPGLEPAVKGEDGQKPPLALEQAADPL 708
        ..A.---A...V........V...H....SLL..G.P.L..P..T.....PR..L.SAQ..T.S. 687
        ....GDPA...V........R...R...SSHL.S..P.HL....GE.V..MP....PQ...T... 703
        .NQS----.SSV........T.LS...L.SDP.K.PP..L...LGL..G.WV.A.PPAD.VPK.F 699

        RDDLGSGIVYSALTCHLCGHLKQCHGQEDGGKVHVVASPCCSCCCEDGSPPMVTPLRAPDAPSSG 773
        ............................EH.EA.T......G...G.R.S.P.S.V..L.P.PG. 752
        V.S...........................QTP.M.....G...G.R.S.PT.......PSPG. 768
        G....F.....S...........H.S..E..QSPI....G.G..YD.R..SLGSLSG.LESCPE. 764

        VPLEASLSPASLALLGVSREGKIPPCLQITPSNVQSSSQTPTAVAMLSPGPACMDTS 830
        .....G..L...GS..L.E.R.PSLFF.PA.G.A.......LT.....T..T.TSA. 809
        .......C.....PS.I.EKS.SSSSFHPA.G.A.......KI.NFV.V..TY.RV. 825
        I.P..N.MS.PKTPSNL.G...-----GP--G----H.PV.SQTTEVPV.ALGIAV. 810
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Fig. 1. Alignment of IL-4Rα-predicted amino sequences from swine (Sw) (GenBank accession no. AY266143), equine (Eq), human (Hu) and murine (Mu). Lines above the sequences represent conserved WSXWS and C[$X_{9/10}$]CXW receptor motifs. Consensus and missing amino acids are represented by "·" and "-", respectively. Amino acids D105 and Y216, denoted by "*", are locations on the human IL-4Rα extracellular domain denoting the centers of two clusters, defining the binding determinants of the receptor with its cognate cytokine (Zhang et al., 2002).

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SwIL13Rα1 MERPARLYGLWALLFCAGG---GGLAAPAETQPPVTNLSVSVENLCTVTWTRNPPEGASPNCSL  61
CaIL13Rα1 ----------------------..V...T...................I..W..........T.  42
HuIL13Rα1 ..W....C......L....GGG..G...T...................I..W.......S....  64
MuIL13Rα1 .A...L.GE.LV..LWTAT--V.QV..AT.V................II..WS.........T.  62

          WYLSHFGNKQDKKITPETHRSEEVPLNERICLQVGSQCSTNESEKPSILVEKCISPPEGDPESA 125
          R.F...D.......A......K.....................DN........TP......... 106
          ..F....D......A...R..I..................................................... 128
          R.F...DDQ.....A.....K..L..D.K..........A.......P..K.............. 126

          VTELQCVWHNLRYMKCTWLPGRNTSPDTNYTLYYWHSSLGQILQCENIYREDQHIACSFALTKV 189
          ..........S.............................K.....D....G...G......NL 170
          .I....I....S....S...................R..EK.H.....F..G.YFG...D.... 192
          ....K.I....S....S...........H......Y...EKSR........G.......K.... 190

          KDSNFDS-SVQIMVKDNAGKIRPAFSIVPSSSHVKPDPPHIKSLSFQNGDLNVQWKNPQNFYSR 252
          ...S.EQH..........R....S.N...LT...........R.F....N.Y............ 234
          ...S.EQH.............K.S.N...LT.R.........N...H.D..Y...E.....I.. 256
          EP.-.EHQN..............SCK..SLT.Y.........H.LLK..A.L.........R.. 253

          CLSYQVEVNNTQAKTHDIFYVEEAKCQNSEFEGNLEGMICFMVPGVLPDTLNTVRIRVKTNKLC 316
          ..........S.TE.N.....................T.....................R..... 298
          ..F.E.....S.TE..NV...Q....E.P...R.V.NTS........................... 320
          ..T.E.......TDR.N.LE...D......SDR.M..TS..QL....A.AVY...V........ 317

          YEDDKLWSNWSQAMSIGQKANPTFYITTLLIIPVIVAAAIIVLLLYLKRLKIIIFPPIPDPGKI 380
          .................ENTD......M..ATQ....G...I..................... 362
          .............E....K.R.S.L...M...V.....D.......................... 384
          FD.N....D..E.Q...KEQ.S...T.M..T...F..V.V.I..F.................... 381

          FKEMFGDQNDDTLHWKKYDIYEKQTKEETDSVVLIESLKKASQ 423
          ...............R.....................N...... 405
          ....................................N...... 427
          ........................S...........N....AP 424
```

Fig. 2. Alignment of IL-13Rα1-predicted amino sequences from swine (Sw) (accession no. AY266142), canine (Ca), human (Hu) and murine (Mu). Lines above the sequences represent conserved WSXWS and C[$X_{9/10}$]CXW receptor motifs. Consensus and missing amino acids are represented by "·" and "-", respectively.

4. Discussion

Receptors play critical roles in modulating host immune responses. As such, proteins engaging the cytokine-binding site of the receptor are being evaluated as a means to polarize host responses to infection and abrogate disease. To this end, comparatively evaluating the primary and secondary structures of less conserved receptors may provide information for developing treatments with unilateral application among disparate species. Within the sequences analyzed, no greater than 0.4% nucleotide sequence variation was observed among any two aligned sequences. This can be attributed predominantly to PCR artifacts when non-proofreading enzymes are used in identifying consensus sequences; however, assignment of consensus sequences does not preclude the possibility that polymorphisms within the IL4R locus may exist within or between swine hosts.

Hage et al. (1999) identified the crystal structure of the human IL-4/IL-4Rα complex where polar binding sites are surrounded and enhanced by the hydrophobic side-chains enveloping the points of contact. Within the hydrophilic core, the critical binding interfaces were localized to amino acids Y13, D72 and Y183 (Hage et al., 1999; Zhang et al., 2002), herein referenced as Y45, D105 and Y216 (Figs. 1 and 3). The investigations further indicated that the hydroxyl groups of Y45 and Y216, and the carboxyl group of D105 are critical determinants for IL-4Rα/IL-4 binding. These data suggest that the observed "species-specificity" of the IL-4/IL-4Rα complex (Mosmann et al., 1987) may be limited to interactions with the mouse orthologue among those depicted in Fig. 1,

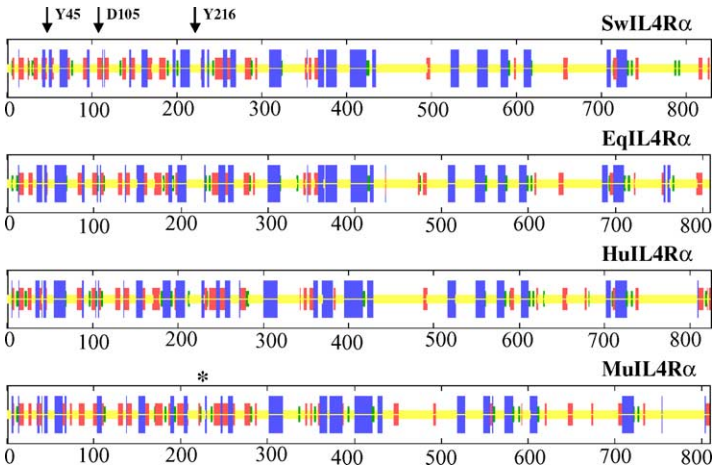

Fig. 3. Predicted secondary structures of the IL-4Rα protein sequences from swine (Sw), equine (Eq), human (Hu) and murine (Mu). Data were analyzed according to an improved version of the self-optimized prediction method (SOPMA) using default parameters. Conformational states are represented as follows: blue = α-helix; yellow = random coil; red = extended strand (sheet); green = β-turn. Arrows depict the locations of amino acids Y45, D105 and Y216 within the extracellular domain of swIL-4Rα within the two clusters defining the binding determinants of the receptor with its cognate cytokine (Zhang et al., 2002). The experimentally determined junction between the extracellular domain and intracellular portion of the MuIL-4Rα is denoted by "*".

given the substitution of the non-polar, L, for Y216 (Fig. 1).

Given the low level of sequence similarity among the IL-4Rα sequences and the absence of crystal structure information on other than the HuIL-4Rα sequence, putative secondary structures of the chains were calculated (Combet et al., 2000) and compared (Fig. 3) to evaluate this character in site recognition. The centers of the two human binding clusters (Hage et al., 1999; Zhang et al., 2002), as well as the location of the junction of the intracellular/extracellular domains are noted in Fig. 3. Surprisingly, given the disparity in sequence homology, primarily in mouse, the overall predicted conformations remained remarkably similar. These data are consistent with secondary structure being evolutionarily conserved and likely playing a role in cytokine binding and cell signaling.

IL-4Rα and IL-13Rα1 mRNAs are expressed in most normal human and murine tissues (Aman et al., 1996; Hilton et al., 1996) and are also expressed on non-lymphoid cells particularly, hepatocytes, epithelial and endothelial cells (Akaiwa et al., 2001). In vitro studies have demonstrated specific cellular expression of these receptors in activated T cells, B cells, and macrophages (Graber et al., 1998; Ogata et al., 1998), although the IL-13Rα1 is reportedly found only on B cells (Pierrot et al., 2001; Andrews et al., 2001). Several lines of evidence suggest a role for these receptors in the development of a Th2-type response: (1) exogenous IL-4 or IL-13 increase both the mRNA and protein expression levels of IL-4Rα and IL-13Rα1 on in vitro activated T cells and B cells (Graber et al., 1998; Ogata et al., 1998); (2) expression of these receptors on activated macrophages is reportedly down-regulated in the presence of IL-4 or IL-13 (Graber et al., 1998), and; (3) Th1 and pro-inflammatory cytokines such as IFNγ inhibit the expression of IL-13Rα1 (Zheng et al., 2003). The importance of regulating IL-4 and IL-13 production in a Th1 or Th2 bias response during protozoan or nematode infections, respectively, has been well-documented (Urban et al., 1991, 1995; Else et al., 1994). Further, studies using chimeric and transgenic mice lacking functional expression of the IL-4Rα, have demonstrated the necessity of the IL-4Rα on non-bone marrow-derived cells for effective immune responses against certain parasites (Urban et al., 2001), and in allergic responses (Kelly-Welch et al., 2004; Nikolaidis et al., 2003). However, to date, no analysis has been performed on expression levels of the IL-4Rα or IL-13Rα1 within swine tissues, in particular, using animals polarized to

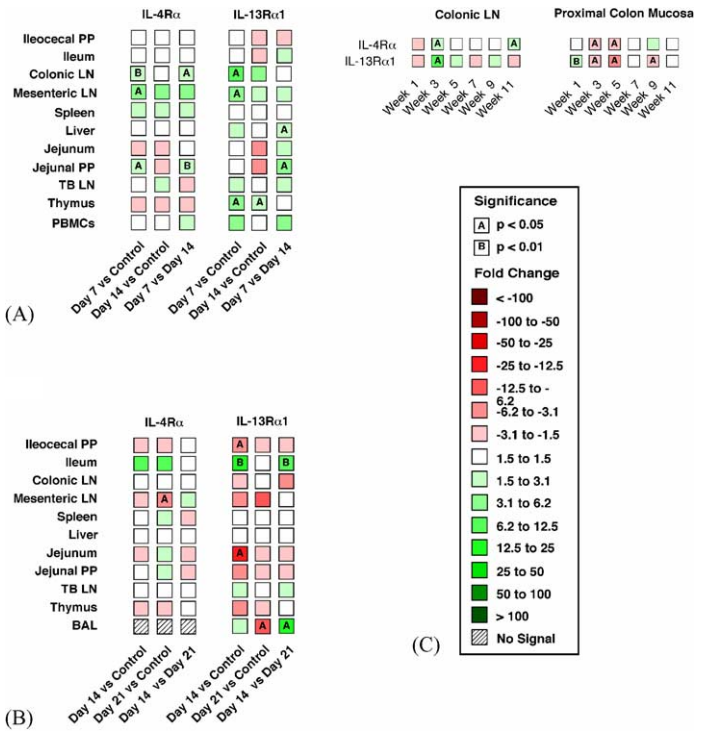

Fig. 4. swIL-4Rα and swIL-13Rα1 expression in various tissues in response to infection with (A) *T. gondii* (Th1-inducing); (B) *A. suum* (Th2-inducing); (C) *T. suis* (Th2-inducing). Pigs were fed a total of 4.5×10^5 oocysts of the VEG strain of *T. gondii*, 1×10^5 infective *A. suum* eggs, or 5×10^3 infective *T. suis* eggs. Real-time PCR analysis was performed on cDNA prepared from the indicated tissue RNA as described in Section 2. The data shown represent the mean fold change in mRNA levels in infected vs. control animals.

a Th1 or Th2 response via experimental infections known to induce these changes.

Data from Dawson et al. (2004a,b) have shown that the animals used in this study exhibited a generalized Th1-pattern of gene expression (significant elevation of IFN-γ and TNF-α mRNA levels) in response to infection with *T. gondii*, while a Th2-pattern (significant elevation of IL-4, IL-5 and IL-13 mRNA levels) was observed in pigs infected with *A. suum* or *T. suis* (Fig. 4). However, regulation of the genes encoding the swIL-4Rα and swIL-13Rα1 receptors, as presented here, seem not to align with conventional wisdom (Graber et al., 1998; Ogata et al., 1998), where receptor mRNA levels were generally elevated in animals infected with *T. gondii* and lower in animals infected with *A. suum*. Several factors may contribute to the unexpected results. First, this deviation may reflect a change in tissue composition and/or local cytokine concentrations due to the migration of effector cells, rather than a change in mRNA-expression levels. Thus, at the site of intense infection, swIL-4Rα and swIL-13Rα1 mRNA levels may be leading indicators of Th1 or Th2 polarization as well as the corresponding return to the non-polarized state. There is a rapid clinical recovery from acute infection with *T. gondii* during the second week of infection that is marked by increased localized production of anti-inflammatory cytokines such as IL-4, IL-13, and IL-10 in the liver, ileum, and MLN (Dawson et al., 2004a) that may be responsible for an increase in expression of IL-4R. In addition, high local concentrations of IL-4 and IL-13 cause decreased intestinal epithelial-cell resistance and sodium-dependent glucose uptake resulting in a state of edema that needs to be corrected in order to establish normal nutrient absorption in the small intestine (Madden et al., 2004). Reduced levels of IL-4Rα expression during the later phase of expulsion of *A. suum* from the intestine may be an effective

way of restoring intestinal homeostasis. Second, our probes do not distinguish between the membrane-bound and soluble forms of the receptors; however, Chilton and Fernandez-Botran (1993) demonstrated the ability of IL-4α to up-regulate the secretion of soluble IL-4Rα in murine splenic cells and further showed parallel transcriptional regulation between the soluble and membrane forms of the receptor (Chilton and Fernandez-Botran, 1997). Finally, as the kinetics of the response were not thoroughly evaluated over the entire period of infection, the times for measuring gene expression may not have been optimal for examining receptor mRNA levels in animals acutely challenged with each organism.

To further evaluate the later possibility and to simplify gene expression analysis of the swIL-4Rα and swIL-13Rα1 mRNAs from a nematode-infected animal, a more holistic time-course evaluation was performed using tissues from *T. suis*-infected animals where the infection is specifically limited to the intestinal tract. This contrasts with the more systemic infection scheme characteristic of migrating *Ascaris* and developing *Toxoplasma* parasites. Upon examining RNA from PCM and CLN, variability in receptor-gene regulation occurred coincidentally with key points in the infection process. Eggs reach the mucosa of the large intestine and hatch into L1 larvae within the first 1–10 DAI, during which up-regulation of swIL-13Rα1 was observed. Over the next several weeks, both the swIL-4Rα and swIL-13Rα1 mRNAs were significantly down-regulated. This takes place as the parasites are tunneling into the mucosa of the proximal cecum and colon, and entering the cellular lining of the Lieberkühn, where they rapidly grow to adults. Substantial inflammation and pathology develops in the surrounding tissues during the cellular penetration phase with concomitant up-regulation of IL-4 and IL-13 mRNA (data not shown). Similar changes in the levels of IL-4 mRNA have been observed in *Ostertagia* infections of bovine as the parasites penetrate the gastric glands lining the abomasum (Almeria et al., 1997).

Given the simultaneous infiltration of bacteria at the site of *T. suis* infections (Mansfield and Urban, 1996) and the localized inflammation that ensues, it is possible that the down-regulation of swIL-4Rα and swIL-13Rα1 mRNAs results from an increase in pro-inflammatory cytokines such as IFN-γ (Zheng et al., 2003); however, no evidence of transcriptional changes in IFN-γ relative to controls was observed during the infection process (data not shown), suggesting that a shift to a localized Th1 type response is not responsible for down-regulating receptor sequences. Furthermore, it has been shown using *T. muris* in a mouse-infection model, that IL-4 is substantially involved in the protective immune response (Else et al., 1994; Curry et al., 1995). The disparity in the regulation between the IL-4 and IL-13 cytokine genes and their corresponding receptors in the PCM may result from variation in the localized concentration of the cytokines and cellular infiltration. This is supported in part by receptor mRNA levels in CLN tissue, which are substantially removed from the immediate site of infection and not subject to the intense host immune responses present in the intestinal mucosa. In this instance, the receptor mRNA levels were more predictive of a Th2 response where significant elevation of both receptor mRNA subsets was observed at 3 weeks post-infection, and the entire course of the swIL-4Rα/swIL-13Rα1 response was essentially the inverse of that observed in the PCM. The rapid and significant down-regulation in the swIL-4Rα and swIL-13Rα1 at the site of infection is consistent with changes in the intracellular signaling cascade that compensate for and are commensurate with, high localized concentrations of the cognate cytokines.

Data presented here demonstrate the importance of studying both biochemical and physiological aspects of receptor/cytokine relationships in deciphering host responses to infection. Understanding interactions involving immune modulation which take place at the cell surface and subsequently effect signal transduction, may offer greater insight into the mechanisms associated with host immune responses than those ascertained by monitoring circulating levels of effector molecules only. Additional experiments are currently underway to clarify the discrepancy between peripheral and localized responses in *T. suis*-infected animals.

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