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Role of β_1 and β_2 subunits of the interleukin-12 receptor in determining T helper 1/T helper 2 responses *in vivo* in the rat

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SUMMARY

Interleukin-12 (IL-12) responsiveness, and hence capacity to mount a T helper type 1(Th1) immune response, may be regulated via differential expression of the IL-12 receptor β_2 subunit at least *in vitro* in human and murine cells. To test whether a similar phenomenon operates *in vivo* in the rat we cloned and sequenced partial cDNAs for rat IL-12Rβ_1 and IL-12Rβ_2 subunits and analysed expression of these genes *in vivo* in two rat strains with different Th1/Th2 bias. After treatment with mercuric chloride ($HgCl_2$), Brown–Norway rats develop Th2-biased autoimmunity whereas Lewis rats do not develop autoimmunity, instead becoming resistant to Th1-biased diseases to which they are normally susceptible. We report close sequence homology between the segments of the rat IL-12R genes sequenced and corresponding mouse genes (95·6% and 92% for IL-12Rβ_1 and IL-12Rβ_2, respectively). Both Brown–Norway and Lewis rats express both β_1 and β_2 subunits of IL-12 receptor *in vivo* in spleen; Brown–Norway rats express the β_2 subunit at a lower level than Lewis rats. After $HgCl_2$ treatment, IL-12Rβ_1 expression was not altered but there was down-regulation of IL-12Rβ_2 expression in both strains. We conclude that relative under-expression of IL-12Rβ_2 by Brown–Norway rats contributes to their Th2 bias, and that down-regulation of IL-12Rβ_2 after $HgCl_2$ administration in Lewis rats underlies subsequent resistance to induction of Th1-biased diseases.

INTRODUCTION

The $CD4^+$ subset of T cells, the T 'helper' (Th) cells can be subdivided according to their cytokine profile. This compartmentalization of Th cells is becoming increasingly complex but at its simplest level there are at least two subsets, Th1 and Th2, that reciprocally regulate one another. Interferon-γ (IFN-γ) production characterizes the Th1 subset while Th2 cells produce interleukin-4 (IL-4).[1] Dominance of either subset may result in disease, for instance Th1 responses are associated with some autoimmune diseases while Th2 responses are associated with allergy and atopy.[2]

IL-12, a heterodimeric cytokine produced mainly by antigen-presenting cells, is essential for the development of Th1 responses.[3] IL-12 acts through specific receptors on both T and natural killer (NK) cells to induce IFN-γ secretion.[4] Two subunits, IL-12 receptor β_1 (IL-12Rβ_1) and IL-12Rβ_2, have been cloned for both human and mouse IL-12R.[5,6] Both subunits belong to the cytokine receptor superfamily. IL-12Rβ_2 by itself binds IL-12 with low affinity but when co-expressed with IL-12Rβ_1 it confers high-affinity binding and IL-12 responsiveness.[7]

Recently it has become clear from *in vitro* studies that human and murine $CD4^+$ T helper cells biased towards a Th2 response are IL-12 'unresponsive' due to selective loss of expression of the β_2 subunit of the IL-12R.[8,9] Expression of the IL-12β_1 subunit remained unaffected. We were interested to test whether this regulatory mechanism operates *in vivo* in the rat, since it could explain many of our observations on the response of different rat strains to mercuric chloride ($HgCl_2$).[10] Certain rat and mouse strains are susceptible to autoimmunity after treatment with low-dose $HgCl_2$, gold, or D-penicillamine. For instance, Brown–Norway (BN) rats treated with low-dose $HgCl_2$ develop an autoimmune syndrome characterized by polyclonal B-cell activation, high circulating immunoglobulin E (IgE) levels, the appearance of IgG1 autoantibodies and widespread tissue injury.[11] In contrast, Lewis rats treated with the same regimen do not develop autoimmunity, instead becoming resistant to diseases to which they are normally susceptible, e.g. experimental autoimmune encephalomyelitis (EAE).[12] The autoimmune syndrome in the BN rat resolves approximately 2 weeks after the first $HgCl_2$ injection and surviving rats are resistant to further induction of disease.[13] The polyclonal B-cell activation and high IgE levels induced in the BN rat by $HgCl_2$ suggested a role for the Th2 compartment

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Table 1. Cytokine gene expression in $HgCl_2$-treated BN and Lewis rats*

	BN	Lewis
IL-2	↑	–
IL-4	↑ ↑ ↑ ↑ ↑	–
IL-6	↑ ↑ ↑	–
IL-10	↑ ↑ ↑	↑ ↑
IL-12	↑	high expression at baseline
IL-13	ND	ND
IFN-γ	↑	–
TGF-β	–	–

Data from ref. 18. ↑, an increase; –, no change; ND, not detected; TGF-β, transforming growth factor-β.

of $CD4^+$ T cells,[14] and this has been confirmed by cytokine gene expression studies. Fully quantitative and semi-quantitative reverse transcription–polymerase chain reaction (RT-PCR) clearly demonstrated that the immune response in $HgCl_2$-treated BN rats is biased towards the Th2 subset with IL-4, IL-6 and IL-10 dramatically up-regulated[15] (Table 1). Previous studies in our laboratory demonstrated that IL-12 gene expression was increased in the BN rat at the time when the $HgCl_2$-induced autoimmune syndrome autoregulates, and that while baseline levels of IL-12 in the Lewis rat are high, they are not further increased by $HgCl_2$.[16]

We wished to test the hypothesis that IL-12 unresponsiveness explained the bias towards a Th2 response in the BN rat. Since the IL-12R had not been cloned in the rat, we cloned partial cDNAs for both the β_1 and β_2 subunits of the rat IL-12R. We then studied IL-12R expression *in vivo* in BN and Lewis rats before and after treatment with $HgCl_2$.

MATERIALS AND METHODS

Rats

BN and Lewis rats were obtained from Harlan UK Ltd (Bicester, UK) and maintained under standard conditions. Splenic RNA was extracted following standard methods.[17]

Cloning and sequencing of partial cDNAs for rat IL-12R β_1 and β_2 subunits

Oligonucleotide primers (Cruachem, Glasgow, UK) for IL-12R β_1 and β_2 subunits were designed based on the sequence of the mouse genes. One microgram of rat splenic RNA was reverse transcribed according to the manufacturer's instructions (Promega, Madison, WI). PCR reactions were performed on a Hybaid thermal cycler, total volume 25 μl, with 3 μl cDNA, 2·5 μl 10*times* PCR buffer, 200 μM dG/T/A/CTP, 0·4 μM each of the forward and reverse primers and 1 unit of Taq polymerase (Bioline, London, UK). PCR conditions were 95° for 5 min (once); 95° for 30 seconds, 53° for 30 seconds, 72° for 30 seconds (30 times); 72° for 5 min for IL-12β_1. The same conditions were used for IL-12β_2 but the primers were annealed at 54°. In the case of both receptor subunits, a band of appropriate size was identified. This band was ligated into pGEM T vector (Promega) and transformed into competent TOP 10F *Escherichia coli* (Invitrogen, Groningen, the Netherlands). Positive colonies were selected by M13 PCR screening and sequenced on an ABI 377 sequencer. Rat-specific IL-12Rβ_2 primers were designed.

Analysis of IL-12R expression in vivo *after $HgCl_2$*

BN and Lewis rats were given $HgCl_2$ (five injections over 10 days, each of 1 mg/kg) by subcutaneous injection. Spleens were removed at various time-points. Total RNA was isolated as above. Expression of IL-12Rβ_1 and IL-12Rβ_2 in $HgCl_2$-treated BN and Lewis rats was analysed using the housekeeping gene β-actin as a control. PCR cycles were kept to a minimum to ensure that the PCR products were analysed within the exponential range of amplification. To further confirm our results serial 1 : 3 dilutions of RT reactions were PCR amplified (data not shown). All primer sequences used are shown in Table 2.

RESULTS

The cDNAs for rat IL-12 β_1 and β_2 show extensive sequence homology with the corresponding mouse genes

The sequences of the partial cDNAs of rat IL-12Rβ_1 and IL-12Rβ_2 are shown below. These sequences have been submitted to the Genebank and have acquired accession numbers as follows: IL-12Rβ_1, AF083328; IL-12Rβ_2, AF083329. The sequence homology with the mouse gene is 95·6% for IL-12Rβ_1 and 92% for IL-12Rβ_2.

IL-12Rβ_1

Rat : tttcgcgtctctgggaagcttc*cc*cagcgtcctcctcgtgggcagtctcg-gatacattgg
Mouse : tttcgcgtctctgggaagcttcgccagcgtcctcctcgtgggcagtctcg-gatacattgg
Rat : cttaaacagggc*t*gcctggcacttgtgcccacc*t*ctgcctac*g*ccctgtg-gcagcactgc
Mouse : cttaaacagggccgcctggcacttgtgcccacccctgcctacaccctgtgg-cagcactgc
Rat : *t*gtggagttccctggcagccagg*a*caagcaggcttggca*a*tggc*g*-caaccctgaggac
Mouse : cgtggagttccctggcagccagggcaagcaggcttggcagtggtg-caaccctgaggac
Rat : ttcccggaggtgttgta
Mouse : ttcccggaggtgttgta

Table 2. Primer sequences used in this study and PCR product sizes

Primer	Sense	Antisense	Size
Mouse IL-12Rβ_1	5′ ccatcattttcgcacactggg	5′ tacaacacctccgggaagtcct	202 bp
Mouse IL-12Rβ_2	5′ tgacagctgctggtgaaagt	5′ atgttggagggtaaatagcc	602 bp
Rat IL-12Rβ_2	5′ ttgcatcgctatcatcgtgg	5′ cctcttttgaagcaataggg	288 bp
Rat β-actin	5′ atgccatcctgcgtctggacctggc	5′ agcatttgcggtgcacgatggaggg	607 bp

IL-12Rβ$_2$

Rat : tgacagctgctggtgaaagtccccaaggaaatgaaagggaattttgtc-cacagggcaa
Mouse : tgacagctgctggtgaaagtccccaaggaaatgaaagggaattttgtc-cacagggcaa
Rat : agccaactggaaa*a*cattcgtgatatcaagcatttgcatcgctat-catc*g*t*g*gtgggcac
Mouse : agccaactggaaagcattcgtgatatcaagcatttgcatcgctatcat-cacggtgggcac
Rat : *t*ttctcaattcgttacttc*a*ggcaaaaggcatttactctcct*t*ctactct-caaaccc*c*aatgg
Mouse : gttctcaattcgttacttccggcaaaaggcatttactctcctgtctactct-caaacctcaatgg
Rat : tat*t*gcagaac*t*attccagatccagcaaa*t*agcacttgggtaaagaag-ta*c*ccc*a*tt*a*tg
Mouse : tatagcagaaccattccagatccagcaaacagcacttgggtaaagaag-tatcccattctg
Rat : gaggagaagatccagc*c*acct*a*t*g*ga*c*aatctcct-gatgg*c*c*t*gg*t*ccgctcctgaag
Mouse : gaggagaagatccagctacctacggataatctcctgatggcatggcc-cactcctgaag
Rat : agcctgagcccctgatcatcaatgaagtcctctaccaaatgatcccag-taggcagacaac
Mouse : agcctgagcccctgatcatccatgaagtcctctaccacatgatcc-cagttgtcagacaac
Rat : cctattgcttcaaaagaggacaagggttccaaggttactc
Mouse : catattacttcaaaagaggccaaggattccaaggctactc

IL-12Rβ$_1$ and IL-12Rβ$_2$ gene expression in $HgCl_2$-treated BN and Lewis rats

Both BN and Lewis rats showed readily detectable expression of both β_1 and β_2 subunits of the IL-12R *in vivo* in the spleen (Figs 1 and 2, day 0, i.e. naive animals). Expression of IL-12Rβ_1 was comparable in the two rat strains (Fig. 1). Expression of IL-12Rβ_2 was markedly greater in Lewis rats compared to BN rats (Fig. 2). After $HgCl_2$ administration, expression of IL-12Rβ_2 was down-regulated in both strains (Fig. 2); expression of IL-12Rβ_1 was not affected by $HgCl_2$ (Fig. 1). In each case, RNA quantity has been corrected for the house-keeping gene β-actin but these data are not shown as IL-12Rβ_1 acts as a control for IL-12β_2 expression.

DISCUSSION

We provide evidence that differential expression of the IL-12Rβ_2 subunit occurs *in vivo* in different rat strains, with the Th2-biased BN rat expressing lower levels than the Th1-biased Lewis rat, and that treatment with $HgCl_2$ leads to selective down-regulation of the IL-12Rβ_2 subunit. The changes in IL-12Rβ_2 subunit expression segregate with susceptibility to Th1/Th2 autoimmunity and may play a role in determining the susceptibility/resistance of the two rat strains to different phenotypes of immune response.

We report that the partial cDNAs which we have isolated for the rat IL-12R β_1 and β_2 subunits show extensive sequence homology with the corresponding mouse genes (95·6% and 92% for IL-12Rβ_1 and IL-12Rβ_2, respectively). Homology between mouse and human IL-12Rβ_1 has been shown to be only 54% at the amino acid level while for IL-12Rβ_2 the human/mouse homology is 68%.[7]

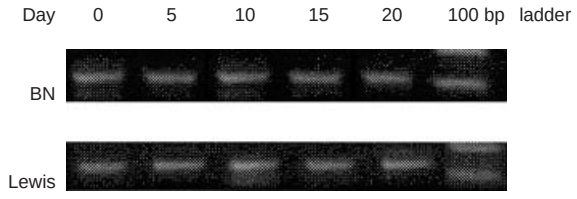

Figure 1. IL-12Rβ_1 expression in BN rats (top panel) and Lewis rats (bottom panel) at days 0, 5, 10, 15 and 20 after the first $HgCl_2$ injection. This figure is representative of three experiments.

Naive BN and Lewis rat strains both express IL-12Rβ_1 and IL-12Rβ_2 in the spleen, so that the preferential Th2 phenotype of immune responses in the BN strain cannot be accounted for by absolute deficiency of IL-12Rβ_2 expression. However, the level of IL-12Rβ_2 gene expression in the spleen was lower in naive BN rats compared to naive Lewis rats, and relative deficiency of IL-12Rβ_2 expression may contribute to the Th2 bias of this strain. Susceptibility to chemically induced autoimmunity is subject to major genetic influences, with loci within the major histocompatibility complex (MHC) and in the cytokine gene cluster on chromosome 10 being strongly linked to susceptibility.[18] Whether these, or other susceptibility loci, exert their effects by regulation of IL-12R expression remains to be determined.

After treatment with $HgCl_2$, IL-12Rβ_2 expression was down-regulated in both rat strains, but IL-12Rβ_1 gene expression was not affected. In BN rats, treatment with $HgCl_2$ leads to massive induction of IL-4 gene expression[15] and this could lead to down-regulation of IL-12Rβ_2. This is compatible with recent data demonstrating that the burst of IL-4 production in BALB/c mice following infection with *Leishmania major* results in down-regulation of IL-12Rβ_2, leading to IL-12 unresponsiveness.[19] However, since a similar degree of down-regulation of IL-12Rβ_2 was seen in Lewis rats, which do not show significant up-regulation of IL-4 in response to $HgCl_2$,[15] other mechanisms may be operating.

The down-regulation of IL-12Rβ_2 expression in Lewis rats treated with $HgCl_2$ is of interest because this treatment is known to render these rats resistant to the induction of EAE, a Th1-biased autoimmune syndrome to which Lewis rats are normally fully susceptible.[11] Loss of IL-12 responsiveness due to down-regulation of IL-12Rβ_2 would provide a plausible mechanism for this resistance. We have previously analysed cytokine gene expression in $HgCl_2$-treated Lewis rats and

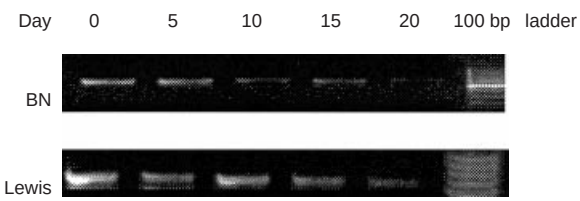

Figure 2. IL-12Rβ_2 expression in BN rats (top panel) and Lewis rats (bottom panel) at days 0, 5, 10, 15 and 20 after the first $HgCl_2$ injection. This figure is representative of three experiments.

shown up-regulation of IL-10.[15] It remains to be seen which of these changes is primary but both may contribute to the induction of a state of resistance to Th1-biased autoimmunity. Further experiments are planned to dissect these regulatory mechanisms.

In conclusion, our results add to the evidence that differential expression and regulation of the β_2 subunit of the IL-12R is an important mechanism whereby the cytokine phenotype of an immune response is determined. Selective means of increasing or decreasing expression of this molecule would have considerable potential for the therapeutic manipulation of desired or undesired immune responses.

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