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The Chicken Leukocyte Receptor Complex: A Highly Diverse Multigene Family Encoding at Least Six Structurally Distinct Receptor Types

Birgit C. Vierlboeck,* Felix A. Habermann,† Ramona Schmitt,* Martien A. M. Groenen,‡ Louis Du Pasquier,§ and Thomas W. Göbel2*

The chicken Ig-like receptors (CHIR) have been described as two Ig domain molecules with long cytoplasmic tails containing inhibitory motifs. In this study, we demonstrate that CHIR form a large family, with multiple members showing great sequence variability among members as well as a great diversity in domain organization and properties of the transmembrane and cytoplasmic segments. We characterize various novel receptor types with motifs indicative of inhibitory, activating, or both functions. In addition to the inhibitory receptors with two ITIM, receptors with a single immunoreceptor tyrosine-based switch motif or receptors lacking a cytoplasmic domain were isolated. Activating receptors with a short cytoplasmic domain and a transmembrane arginine assembled with the newly identified chicken FceRIγ chain. Three bifunctional receptor types were characterized composed of one or two C2-type Ig-like domains, a transmembrane region with a positively charged residue and combinations of cytoplasmic motifs such as ITIM, immunoreceptor tyrosine-based switch motif, and YXXM. RT-PCR revealed distinct expression patterns of individual CHIR. All receptor types shared a conserved genomic architecture, and in single Ig domain receptors a pseudoxon replaced the second Ig exon. Southern blot analyses with probes specific for the Ig1 domain were indicative of a large multigenic family. Of 103 sequences from the Ig1 domain of a single animal, 41 unique sequences were obtained that displayed extensive variability within restricted Ig regions. Fluorescence in situ hybridization localized the CHIR gene cluster to microchromosome 31 and identified this region as orthologous to the human leukocyte receptor complex. The Journal of Immunology, 2005, 175: 385–393.

Inhibitory receptors have been the focus of intensive research in the past years (1, 2). They can be grouped into type I transmembrane molecules belonging to the Ig family and in C-type lectin family members. The unifying feature of these receptors is their ability to attenuate cellular activation by cytoplasmic ITIM, a 6-aa sequence composed of (I/V/L/S)-X-Y-X-(L/V), in which X denotes any amino acid (3). The ligation of the receptors leads to Src family kinase-mediated tyrosine phosphorylation and successive recruitment of Src homology domain phosphatases, such as Src homology region 2 domain-containing phosphatase-1 (SHP-1),3 SHP-2, and SHIP. These phosphatases dephosphorylate a number of intracellular substrates and neutralize stimulatory signals mediated by activating receptors (4, 5).

Certain receptors such as KIR2DL5, 2B4, CD150, and Ly-9 have a cytokine immune receptor tyrosine-based switch motif (ITSM), a modification of the ITIM, in which the first amino acid is replaced by a threonine. The ITSM recruits both the signaling lymphocyte activation molecule-associated protein signaling lymphocyte activation molecule-associated protein and SHP-2 (6, 7).

Inhibitory receptors are frequently expressed on the cell surface in combination with activating counterparts that share a highly homologous extracellular domain, but lack the cytoplasmic ITIM. Instead, a positively charged transmembrane residue such as a lysine present in most activating killer Ig-like receptors (KIR) or an arginine found in the leukocyte Ig-like receptors (LILR) mediates the association to adaptor molecules that transduce signals into the cell after ligand binding. These adaptors are shared by a wide range of cell surface receptors such as the TCR and FcR. The activating KIR associate with the DAP12 signaling molecule, whereas the LILR use the FcεRIγ chain (8). The pairs of inhibitory and activating receptors may recognize different ligands, but they can also bind highly related ligands, and the cellular response is determined by the strength of the opposing signals.

KIR2DL4 and NKP44 are unique primate NK cell receptors because they have a positively charged transmembrane residue and a single cytoplasmic ITIM. KIR2DL4 is expressed on all NK cells, and it has been shown to bind to the nonclassical HLA-G molecule expressed on fetally derived trophoblast cells (9). Although KIR2DL4 is capable of inducing cytotoxicity in previously activated NK cells, in resting NK cells it only triggers IFN-γ secretion (10). Mutations of the charged transmembrane residue converted KIR2DL4 to an inhibitory receptor (11). NKP44 is associated with the disulfide-linked homodimeric adaptor protein DAP12, and it is only expressed by activated NK cells. It has been demonstrated that the presence of the cytoplasmatic ITIM does not inhibit the NKP44-mediated NK cell activation (12, 13).
In humans, various Ig-like inhibitory receptor families are found at different chromosomal locations. The leukocyte receptor complex (LRC; human chromosome 19q13.4) comprises several families, such as the LILR, KIR, leukocyte-associated Ig-like receptors, and the natural cytotoxicity receptor Nkp46 (14, 15). The triggering receptors expressed by myeloid cell gene cluster with the NKP44 gene are located on chromosome 6—10 megabase pair from the MHC class II region (16), and the related CMRF35 cluster is found on chromosome 17 (17). These families contain multiple activating and inhibitory receptors with variable numbers of Ig-like extracellular domains. The different types of the Ig domains present in the various receptors allow further classification into single V-type receptors (NKP30, NKP44), C2-type receptors (LILR, KIR), and combinations like in Siglec (N-terminal IgV and multiple IgC2) (18).

The characterization of homologous gene families in other nonmammalian vertebrates has been limited to the fish and the chicken. Novel immune type receptors have been analyzed in detail in various fish such as pufferfish, zebrafish, and channel catfish. These receptors have a unique combination of an IgV and a V-like IgC2 domain and differ in this aspect to all receptors found in mammals. The novel immune type receptors show extraordinary variation with multiple activating and inhibitory family members (19, 20). The function and potential ligands of these receptors are largely unknown.

Recently, five chicken Ig-like receptor (CHIR) have been analyzed in the chicken. All of them had two IgC2 domains and could be further classified into four inhibitory receptors (CHIR-B1 to CHIR-B4) and a single putative activating receptor CHIR-A (21) that has a positively charged histidine residue located at an atypical location in the transmembrane domain. The detailed analysis of the inhibitory receptor CHIR-B2 indicated that this receptor is mainly expressed on B lymphocytes and inhibits proliferation by highly conserved signaling mechanisms (22).

In this study, we extend the analyses of the CHIR family by characterizing novel receptor types, including a typical activating receptor and a novel inhibitory receptor form that has an ITSM. Characterizing novel receptor types, including a typical activating and an inhibitory receptor form that has an ITSM, reveals an enormous degree of variability. These data suggest that CHIR represent a unique receptor family with multiple diversified functions.

Cell preparations and cloning procedures

Lymphocytes from bursa, thymus, spleen, and blood were prepared using standard procedures. T cells were activated with Con A by exposing splenocytes to 10 μg/ml Con A for 24 h and harvested after 72 h. Intestinal intraepithelial lymphocytes were prepared as described before (24), and DNA PDS were FACs sorted from splenocytes and expanded in vitro with chicken rIL-2 (25).

Cellular total RNA was prepared using either TRIzol (Invitrogen Life Technologies) or Absolutely RNA RT-PCR Miniprep kit (Stratagene), and cDNA synthesis was performed with the Revert H Minus First Strand cDNA Synthesis kit (MBI Fermentas). For cloning, Herculase Enhanced DNA Polymerase (Stratagene) was used for PCR at 2 min of denaturation at 95°C, 35 cycles of 10 s at 95°C, 30 s at primer specific temperature, 2 min at 72°C, and a final extension time of 10 min at 72°C. For PCR expression analyses, TaqDNA Polymerase (Brinkman Instruments) was used at same conditions, but only 30 cycles of amplification. Primer sequences and their specific temperatures for cloning and expression analyses are summarized in Table I. PCR products were cloned into a pcDNA3.1/V5-His TOPO Vector (Invitrogen Life Technologies); colonies were screened by PCR; and plasmids from positive colonies were isolated using the NucleoSpin Plasmid Kit (Macherey-Nagel) and sequenced (GATC). Deducing amino acid sequences were further analyzed using PSORT (http://psort.nibb.ac.jp/form2.html) and PSIpred (http://bioinf.cs.ucl.ac.uk/psipred/) (26) for secondary structure prediction. The sequence variability of the Ig1 domain was calculated using the Shannon Entropy function (27) (http://info.gmd.de/infodatavt/Fsoot.html) (26) for secondary structure prediction. The sequence variability of the Ig1 domain was calculated using the Shannon Entropy function (27) (http://info.gmd.de/infodatavt/Fsoot.html) (26) for secondary structure prediction. The sequence variability of the Ig1 domain was calculated using the Shannon Entropy function (27) (http://info.gmd.de/infodatavt/Fsoot.html) (26) for secondary structure prediction. The sequence variability of the Ig1 domain was calculated using the Shannon Entropy function (27) (http://info.gmd.de/infodatavt/Fsoot.html) (26) for secondary structure prediction.
tetramethylrhodamine TAMRA-dUTP (Applied Biosystems). The cycling profile was 15 min at 95°C, 25 cycles with 60 s at 94°C, 60 s at 60°C, and 120 s at 72°C. Amplification products were partially digested with DNase (Sigma-Aldrich) to an average fragment size of 150 bp. To identify the chicken chromosome carrying CHIR-A2, the gene fragment was cohybridized with two BAC probes in different colors: bacterial artificial chromosome (BAC)-DNA from clones WAG-87P2 and WAG-27N2 were labeled with two BAC probes in different colors: bacterial artificial chromosome (BAC)-DNA from clones WAG-87P2 and WAG-27N2 were labeled with biotin-dUTP (Roche) and DNP-dUTP (Applied Biosystems), respectively, by standard nick translation.

Individual probes were mixed and ethanol precipitated with 20-fold excess of chicken cot-1-DNA and resuspended in hybridization buffer containing 50% (v/v) formamid, 2× SSC, and 10% dextran sulfate. Final concentration of each probe was 10 ng/μl.

The probe mixture was denatured at 80°C for 5 min and cooled on ice until application to the slides. Probes were hybridized to metaphase spreads from normal embryonic chicken fibroblasts. Before FISH, metaphase spreads were stained with 4′,6-diamino-2-phenylindole (DAPI) and imaged with an epifluorescence microscope equipped with a Plan-Apochromat 100×/1.4 oil immersion objective (Carl Zeiss, Jena, Germany) and a cooled charge-coupled device camera (AxioCam; Carl Zeiss) using Axiovision software. Images were overlaid to a red, green, blue image, assigning a false color to each channel.

**Results**

**Novel CHIR genes encoding activating, inhibitory, and bifunctional receptors**

Table I. Oligonucleotides

<table>
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<tr>
<th>No.</th>
<th>Sequence</th>
<th>Specificity</th>
<th>Location</th>
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<tr>
<td>474a</td>
<td>GGATCC TACGACAGCTGGCACGTTGGCCCTTC</td>
<td>CHIR</td>
<td>SP (I)</td>
</tr>
<tr>
<td>453a</td>
<td>AGACCTTAAACTCTTCTCCGCCAGC</td>
<td>CHIR-B6</td>
<td>CY (V)</td>
</tr>
<tr>
<td>509a</td>
<td>GATATCGAGGCTGGCCGTTG</td>
<td>CHIR-A2</td>
<td>CY (V)</td>
</tr>
<tr>
<td>551a</td>
<td>GATATCGAGGCTGGCCGTTG</td>
<td>CHIR-AB1/AB2</td>
<td>Igl (III)</td>
</tr>
<tr>
<td>574a</td>
<td>GACACCAGCGACACTGGCAC</td>
<td>CHIR-B4/B5</td>
<td>CY (VII)</td>
</tr>
<tr>
<td>554a</td>
<td>GGATCC TACGACAGCTGGCACGTTGGCCCTTC</td>
<td>FeCyR1y chain</td>
<td></td>
</tr>
<tr>
<td>555a</td>
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<td>FeCyR1y chain</td>
<td></td>
</tr>
<tr>
<td>306a</td>
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<td>MHC signal peptide</td>
<td></td>
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<tr>
<td>694a</td>
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<td></td>
</tr>
<tr>
<td>693a</td>
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<td>CHIR-A2-V5 construct</td>
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<td>658a</td>
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<td>663a</td>
<td>GAGGAGAAGTGGAGATGTCG</td>
<td>Igl domains</td>
<td>Igl (III)</td>
</tr>
</tbody>
</table>

*a* and *b* indicate sense and antisense primers, respectively.

* The location of the primers (exons in parentheses) is indicated.

* CY, cytoplasmic; TM, transmembrane.

The EST database search for CHIR genes allowed the identification of highly conserved regions in the CHIR genes suited for RT-PCR amplification. Several cDNAs derived from PBL, spleen, and in vitro IL-2-expanded CD3−/CD8+ cells were amplified using a set of CHIR-specific primers (Table I). By cloning and sequencing, five novel CHIR types were identified*4* (Fig. 1A). CHIR can be grouped into inhibitory receptors (CHIR-B) with a long cytoplasmic domain containing ITIM, activating receptors (CHIR-A) with a short cytoplasmic tail, and a positively charged transmembrane region as well as bifunctional receptors (CHIR-AB) combining inhibitory and activating features. All isolated CHIR gene were germlinal center (GC) rich with ~60% GC content.

The extracellular domains of CHIR-A, CHIR-B, and CHIR-AB3 genes have two C2-type Ig domains, while CHIR-AB1 and CHIR-AB2 have only a single Ig domain. The membrane-distal Ig1 domain and the membrane-proximal Ig2 domain of a given CHIR share only ~20–25% amino acid identity. In contrast, the separate comparison of all CHIR Ig1 and of all CHIR Ig2 domains revealed 60–90% amino acid identity.

Features of the transmembrane region can be used to further classify the different CHIR. A positively charged amino acid (arginine or lysine) was found in a typical position in all CHIR-A and CHIR-AB, which most likely mediates the association to an adapter molecule. Except CHIR-AB3, all CHIR had a conserved transmembrane cysteine residue close to the extracellular domain with the potential to mediate dimerization, which we have already demonstrated for CHIR-B2 (22). The analysis of the periodicity of the α-helical transmembrane region showed that the transmembrane cysteine is situated opposite to the charged arginine or lysine, indicating that a cysteine-mediated dimerization would not interfere with the association of an adapter molecule to the charged residue

* The sequences presented in this article have been submitted to GenBank under the following accession number(s): AF306851, CHIR-A1; AJ745093, CHIR-A2; AJ639839, CHIR-B4; AJ879908, CHIR-B5; AJ879910, CHIR-B6; AJ745094, CHIR-AB1; AJ745095, CHIR-AB2; AJ879909, CHIR-AB3; AJ745098, CHIR-A2 genomic; AJ879911, CHIR-B4 genomic; AJ745097, CHIR-AB1 genomic; AJ745096, CHIR-AB2 genomic; CHIR-Ig1 to CHIR-Ig4; AJ879951 to AJ879991.
In contrast, the previously characterized CHIR-A1 molecule lacks the conserved lysine or arginine, but has a positively charged histidine located 16 aa below the conserved cysteine (21). This would place both the cysteine and the histidine to the same side of the α-helix, preventing either the dimerization or the association with an adaptor molecule. CHIR-A1 resembles in many aspects CHIR-B6 (Fig. 1A), both of which lack an extended cytoplasmic region and a prototypic charged transmembrane residue.

The formerly identified inhibitory receptors CHIR-B1 to CHIR-B4 all displayed two cytoplasmic ITIM (22); however, in CHIR-B5 an N-terminal ITIM and a C-terminal ITSM were found (Fig. 1A). CHIR-AB1 and 2 both contain a long cytoplasmic region with a single ITIM located at the position of the C-terminal ITIM of CHIR. Inspection of the position of the N-terminal ITIM revealed a switch to a methionine at position related to the conserved tyrosine that generated a YXXM motif, which is found in activating signal molecules such as DAP10. Finally, CHIR-AB3 displayed an ITIM and ITSM in combination with a positive transmembrane residue.

Mining of chicken EST databases revealed that several clones with significant nucleotide sequence similarity to CHIR genes have been sequenced (30). The EST clone Riken_19p13 (accession AJ720544) resembles a full-length homologue of CHIR-AB1 sharing 96% nucleotide identity. It is composed of a 33-bp 5' untranslated, a 657-bp coding sequence, and a 585-bp 3' untranslated that lacks any typical polyadenylation signal (AATAAA or ATTAAA), but has several potential sites of the consensus NNTANA (31).

Collectively, by sequence analyses, CHIR can be classified into at least six different forms, including inhibitory receptors with ITIM and ITSM, receptors lacking a cytoplasmic domain, activating type receptors with positively transmembrane residues, and multiple forms with one or two Ig domains, charged transmembrane residues, and a combination of YXXM, ITIM, and ITSM.

**CHIR are expressed in different leukocytes**

The cell type-specific expression of the various known Ig-like receptors differs markedly. Some families such as KIR are restricted to NK cells and CTLs. In contrast, LILR are expressed by a wide range of myeloid and lymphoid cells. To evaluate the cell type-specific expression pattern of CHIR, their nucleotide sequences were aligned, and regions with low sequence similarity were selected to design primers that were specific for seven individual CHIR genes (Table I). These primers were used in RT-PCR of RNA from freshly isolated cells of chicken lymphoid organs, in vitro IL-2-expanded cells, and several cell lines (Fig. 2). Amplification of β-actin served as a control of cDNA integrity, and CHIR-containing plasmids were included to demonstrate the size of the amplicon. Each of the analyzed genes showed a different
The expected size of the PCR product is indicated. A positive control using cloned CHIR genes was included to differentiate between amplification of cDNA as opposed to genomic DNA (upper band in CHIR-B5). β-Actin amplification served as cDNA quality control. Cell lines included macrophage lines (BM-2, HD11), B cell lines (2D8, RP-9), and a T cell line (UG-9).

RNA expression pattern. Except CHIR-AB2, which was only detected in the macrophage cell line BM-2, all other CHIR genes were found to be expressed in several tissues and cell lines (Fig. 2). The CHIR-B5-specific primers amplified two DNA species: the 500-bp product observed in several tissues was obtained from genomic DNA (as verified by sequencing), whereas the 420-bp product was obtained from cDNA. Notably, no RNA expression of any of the CHIR genes could be detected in bursa and thymus. These analyses provide evidence for restricted expression patterns of individual CHIR.

CHIR-A2 associates with the FcεRIγ chain

The positively charged transmembrane residue present in CHIR-A and CHIR-AB suggested that these receptors interact with adaptor molecules such as DAP12 or FcεRIγ. Database mining allowed us to identify an EST clone (accession BQ484203) with high sequence similarity to the human FcεRIγ chain. The deduced amino acid sequence of the chicken FcεRIγ gene shares ~60% identity with the human homologue. The chicken DAP12 homologue could not be found in the databases. Because the adaptor molecules are usually conserved and allow cross-species interaction (32), the human DAP12 gene was used in the assays instead of the chicken gene.

The adaptor molecules were tagged with a FLAG epitope to check for expression after transfection, while a V5 epitope was attached to CHIR-A2 for surface staining. Single transfection of CHIR-A2-V5 into 293-T cells did not induce surface expression of CHIR-A as determined by negative anti-V5 staining. Following cotransfection of CHIR-A2-V5 together with FcεRIγ-FLAG, the anti-V5 mAb detected ~19% of surface-positive cells (Fig. 3; mean fluorescence intensity 53 as compared with 14 in single transfections). In contrast, the DAP12-FLAG construct was unable to restore surface expression of CHIR-A2 (Fig. 3). Both adaptors were expressed well in the 293-T cells, as detected by anti-FLAG control staining (data not shown). Similar experiments were performed with CHIR-AB1 and CHIR-AB2; however, both of these molecules were expressed on the cell surface without cotransfection of an adaptor molecule, and the expression levels were not increased after cotransfections (data not shown). In summary, CHIR-AB seems to be expressed without DAP12 or FcεRIγ; CHIR-A2 is associated with the FcεRIγ chain.

Single Ig domain CHIR have a pseudoexon instead of the Ig2 domain

As a next step in the analysis of CHIR, PCR on genomic DNA was performed to characterize the genomic structure of the different CHIR types. The genomic sequences isolated shared between 94 and 98% identity with the cDNA sequences. All analyzed CHIR genes displayed a highly conserved exon-intron organization (Fig. 4). The signal peptide was encoded by two exons; each Ig domain was located on a separate exon, followed by a transmembrane exon, and in the case of CHIR-B and CHIR-AB two cytoplasmic exons. All exon-intron boundary sequences followed the gt-ag rule. The cytoplasmic exons were in phase 0, whereas all the others were in phase 1. Due to the relatively short introns, the entire length of the inhibitory CHIR genes was ~2000 bp, while the activating CHIR genes lacking the cytoplasmic exons were ~1200 bp in size. Both exon and intron lengths were found to be highly conserved (Fig. 4).

CHIR-AB1 and CHIR-AB2 genes have a pseudoexon in place of exon 4, and therefore encode only a single Ig domain, which is most homologous to the membrane distal Ig domain of CHIR-A and CHIR-B. These pseudoexons are characterized by multiple mutations, such as frame shifts that are caused by alternative splicing sites, single nucleotide deletions before the triplet encoding the first conserved cysteine, and nucleotide insertions before the second conserved cysteine. These analyses collectively show that the CHIR resemble compact genes with highly conserved exon-intron structures that are similar to other mammalian Ig-like receptors.

CHIR belong to a large, highly polymorphic family

The analyses of the CHIR genomic sequences revealed 94–98% identity with the cDNA sequences, and suggested the existence of multiple CHIR genes and/or various allelic forms is present in the chicken genome. To further substantiate this hypothesis, Southern blots with DNA samples from single animals of four different chicken lines were performed (Fig. 5A). The 32P-labeled probe specific for the Ig1 domain of CHIR-A2 hybridized with multiple
DNA fragments (14–24) even under highly stringent conditions. Besides common hybridization patterns found in all four chicken lines, there were multiple line-specific bands, indicative of the polymorphic nature of the CHIR gene locus.

The Southern blot analysis provided further evidence for the existence of a large number of CHIR genes in the chicken genome. Next, a primer pair was designed that binds to conserved parts in the Ig1 domain. This primer pair indeed amplified the Ig1 domain...
of all cloned CHIR genes as verified on plasmid DNA (data not shown). RNA from a single M11 chicken (B2 haplotype) was isolated from PBL using a reagent kit that digests DNA to exclude genomic DNA contamination. RT-PCR amplification, plasmid cloning, and sequencing of >100 clones revealed 41 unique sequences, which were further analyzed by calculating the variability using Shannon entropy (Fig. 5B). The variability was plotted against the predicted secondary structure of the CHIR, which follows a C2-type Ig-fold with typical areas of Ig β strands. Areas of highest variability were particularly present between the c and e strand and after the f strand (Fig. 5B).

In conclusion, a large number of different CHIR mRNAs encoded by multiple genes that are characterized by variation hot spots in the Ig1 domain is expressed in individual animals.

**CHIR genes are located on microchromosome 31, which is orthologous to the human LRC region**

Recently, a first draft of the chicken genome has been published (33). Searching the chicken genome database with various CHIR sequences revealed a large number of CHIR entries; however, none of them assigned to a chromosome (33). Genome assemblies based on whole genome shotgun sequencing of the chicken microchromosome 31 could not be assembled, and consequently, all of the CHIR are not assigned to a chromosome (33). Genome assemblies based on whole genome shotgun sequencing of the chicken microchromosome 31 could not be assembled, and consequently, all of the CHIR are not assigned to a chromosome (33).

**Discussion**

In this study, we characterize five novel members of the CHIR family, including activating, inhibitory, and bifunctional receptors. Although some CHIR have prototypic ITIM or short cytoplasmic domains, as found in typical inhibitory and activating receptors, respectively, others have variable cytoplasmic tails, such as a combination of ITIM and ITSM, like in KIR2DL5 (36), or an uncharged transmembrane region with a short cytoplasmic tail.

The CHIR-AB represent a novel receptor type, which combines a positively charged transmembrane region and various cytoplasmic motifs. Primate KIR2DL4 and NKp44 are the only receptors identified to date that also combine a positively charged transmembrane residue with a single cytoplasmic ITIM (9, 12). In both cases, these receptors seem to be activating rather than inhibitory (10, 13); however, their exact function remains elusive. The multiple CHIR-AB genes are particularly interesting because they are characterized by various combinations of signaling motifs such as ITIM, ITSM, and particularly an YXXM motif, which is present in the DAP10 adaptor molecule and forms a potential SH2 domain binding site for the p85 unit of the PI3K (37).

CHIR-B6 has striking similarity to the previously characterized CHIR-A1 (21), both of which lack a cytoplasmic domain and a prototypic positively charged transmembrane residue. They are most likely generated by introducing a premature stop codon located in intron 5 that has not been spliced correctly. The EST clone BX262283 provides further evidence for this hypothesis, because it has an identical structure encoding the entire intron 5, including the stop codon, followed by the correctly spliced exons 6 and 7.

The CHIR gene family is located on a chromosome region, which is orthologous to the human LRC region on chromosome 19q13.4. Data regarding the entire chicken LRC and adjacent genes would be informative; however, in the first draft of the chicken genome project, the chicken microchromosome 31 could not be assembled, and consequently, all of the CHIR are not assigned to a chromosome (33). Genome assemblies based on whole genome shotgun sequencing of the chicken microchromosome 31 could not be assembled, and consequently, all of the CHIR are not assigned to a chromosome (33).
genome shotgun data are known to frequently miss repetitive regions with gene families of highly related genes such as the CHIR gene cluster. The same effect is seen for the MHC gene cluster on chromosome 16, which also is mainly found in the ChrUn section of the assembly. In addition, it was shown (33) that the regions with a high GC content also appeared to be underrepresented in the chicken genome assembly, which probably is a second reason that chromosome 31 and the CHIR cluster are missing from the assembly.

The human LRC spans ~1 Mb on chromosome 19q13.4 (14), a size typical of chicken microchromosomes. Intensity and size of the FISH signal suggest a large number of CHIR genes covering the microchromosome to a great extent. This may indicate that the CHIR gene cluster occupies the entire microchromosome. To date, we do not know how many different CHIR genes exist in the chicken. The analysis of mRNA from PBL from a single animal revealed the expression of 41 different CHIR Ig1 domain sequences in a single animal, and the number of CHIR genes may considerably vary between individuals, as indicated by Southern blot analysis comparing genomic DNA from individuals from different chicken lines. These results fit well to the mammalian LRC, in which the KIR genes are known to evolve very rapidly even within a species (38, 39). Once the entire cluster has been sequenced and analyzed in detail, it may be necessary to rename the CHIR according to the human genome organization nomenclature as “leukocyte Ig-like receptor” (40).

The mammalian LRC encodes multiple distinct families of Ig-like inhibitory receptors, such as KIR, LILR, and leukocyte-associated Ig-like receptor (14). However, the low overall sequence identity of the CHIR to these mammalian receptor families does not allow simply clarifying the common ancestry. The high degree of variability and the presence of two Ig domains closely resemble the KIR situation, while LILR are less variable and display multiple Ig domains. Vice versa the expression pattern in different leukocytes, and the association with the FcεRIβ chain are features found in LILR, but not KIR. Interestingly, the positive transmembrane arginine residue in CHIR is only encoded by the AAG triplet, which is identical with all primate KIR2DL4 and both bovine KIR2DS1, KIR3DS1, whereas all LILR use the CGC codon. The presence of a pseudoxon instead of a functional Ig exon is another feature of KIR, but not LILR (41).

In an attempt to obtain more information regarding the CHIR structure, we used comparative modeling of the tertiary structure with SWISS-MODEL (Fig. 7). Strikingly, these programs revealed a folding that is similar to the LILRB1 structure (42). In particular, α-helical secondary structures as observed in LILRB1 (pdb entry code 1G0X) are also modeled between the c, e, and f β-strands in the CHIR Ig1 domain (Figs. 1 and 7). The region between the c and e β-strands seems to be particularly variable based on the Shannon entropy analysis and may thus be important for ligand binding, as has been demonstrated for LILRB1 to be the responsible area for binding to MHCII α3 and to UL-18, a viral MHC-like molecule (43).

Although the CHIR ligands are currently unknown, some CHIR may bind to MHC class I molecules, like most KIR and certain LILR. The chicken minimal essential MHC is characterized by low numbers of different MHC class I molecules expressed, which may lead to the high susceptibility of some chicken strains to viruses (44). The CHIR repertoire on NK cells in combination with the MHC class I molecules expressed could be decisive for the susceptibility of different chickens against viral infections. Moreover, it is tempting to speculate that in addition to physiological CHIR ligands such as MHC class I or related proteins, the CHIR system may also be exploited by viral proteins as an immune evasion mechanism (45). This has been especially well documented for herpesviruses and the chicken Marek’s disease virus, as a member of the α-herpesviruses may provide a source for viral ligands (46). It is interesting to note that T cell lines that have been transformed with the Marek’s disease virus are resistant to NK cell lysis (47), thus suggesting that they may express virus-encoded ligand that triggers inhibitory NK cell receptors.

As indicated by sequence characteristics, structural modeling, and expression patterns, the CHIR family displays features of both KIR and LILR. The CHIR gene family is characterized by a modular architecture combining great diversity in the extracellular Ig domain with different cytoplasmic domains that creates a unique multigene family with novel types of receptors that have not been identified in mammals. Future studies will now address individual CHIR, their ligands, cell-specific expression, and their functional role during immune responses.

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References


