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Role of Chicken TL1A on Inflammatory Responses and Partial Characterization of Its Receptor

Tetsuya Takimoto,* Kan Sato,† Yukio Akiba,* and Kazuaki Takahashi1*

The role of chicken TNF-like ligand 1A (ChTL1A) on inflammation and its receptor candidates was investigated to further understand its function as a proinflammatory cytokine. ChTL1A decreased the viability of CHO-K1 cells transfected with chicken TNFR2 or decay receptor 3 and bound to TNFR2 and decay receptor 3. ChTL1A was detected in chicken blood samples taken 4 h after LPS injection. Increased mRNA for inflammatory response-related factors such as IL-1β, IL-6, ChTL1A, IFN-γ, inducible NO synthase, and cyclooxygenase 2 were found in spleen samples following LPS injection. Ceruloplasmin and α1 acid glycoprotein (as positive acute phase proteins) were increased in chicken plasma 12 h after ChTL1A injection. The injection of anti-ChTL1A Ab was able to prevent typical increases in plasma nitrite plus nitrate, ceruloplasmin, and α1 acid glycoprotein concentrations following LPS injection. These results indicate that ChTL1A is a proinflammatory cytokine in chickens, animals that do not have TNF-α and lymphotoxin α orthologous genes, and that its proinflammatory action is, at least in part, expressed through binding to TNFR2. The Journal of Immunology, 2008, 180: 8327–8332.

T he TNF ligand superfamily of proteins, including TNF-α, consists of 19 members and their receptors, namely, the TNF superfamily of proteins has 29 members (1). Binding of TNF ligands to their related receptors results in a large variety of signals within cells and plays important roles in inflammatory responses, osteogenesis, hematopoiesis, and morphogenesis. Particularly in mammalian inflammation, TNF-α acts as a proinflammatory cytokine that induces the release of a diverse range of cytokines, including other proinflammatory cytokines such as IL-1 and IL-6 (2, 3). Following the production of these proinflammatory cytokines, synthesis of both NO and PGE2 is up-regulated due to enhancement of inducible NO synthase (iNOS)2 and cyclooxygenase 2 (COX-2) expression (4, 5).

TNF-like activity has been detected only in the supernatant of chicken macrophage culture medium (6), to date, and molecular cloning of TNF-α has been unsuccessful in avian species. We previously cloned chicken TNF-like ligand 1A (ChTL1A), which has been shown to be a homolog of human TL1A (TNFSF15) and belongs to the TNF ligand superfamily (7). We also observed that ChTL1A mRNA expression was enhanced following LPS stimulation, particularly in chicken spleen, and recombinant ChTL1A protein in culture caused cell death of both the L929 cell line and chicken primary fibroblast (7). In addition, Park et al. (8) reported that the expression of ChTL1A in the spleen, liver, and intestinal intraepithelial lymphocytes was enhanced by *Eimeria* infection and that ChTL1A induced chicken tumor cell death. These reports suggest that ChTL1A has TNF-α-like functions. Although i.v. injection of recombinant ChTL1A to chickens decreased feed intake, increased rectal temperature, and plasma nitrite plus nitrate concentrations (7), there is little information available addressing whether ChTL1A induces other proinflammatory cytokines and subsequent inflammatory reactions.

Most TNF ligands identified to date bind to a single receptor, while a few can bind to more than one. It has been reported that TNF-α signaling in mammals is transduced predominantly through TNFR 1 and 2, while TL1A binds to a decoy receptor (DcR) 3 and death receptor (DR) 3 (1). No cell type in the body has yet been found that does not express TNFR1, whereas expression of TNFR2 is mainly limited to immune cells and endothelial cells. Mammalian TL1A shows TNF-α-like activity, e.g., modulation of T cell activation and apoptosis of hematocytes due to activation of NF-κB through DR3 (9). However, it is not clear which receptors bind ChTL1A.

The present study was conducted to characterize the receptor for ChTL1A and to obtain further evidence whether ChTL1A plays an important role in chicken inflammatory responses as a functional homolog of TNF-α.

Materials and Methods

Preparation of recombinant ChTL1A and antiserum

The full-length open reading frame (ORF) of ChTL1A cDNA was amplified by PCR using the following primer set: forward primer flanked by EcoRI 5′-GAA TTC ATG GAT CAC GGG GCT GAA ATA ACC-3′, reverse primer flanked by XhoI 5′-CTC GAG CAT CAC GGG GCT GAA ATA ACC-3′, PCR product was digested and ligated into the multicloning site of pGEX-5X-1 (Amersham Biosciences) and vector transformed into *Escherichia coli* BL21 (Novagen). Positive clones were selected by ampicillin. *E. coli* were induced to produce fusion proteins by 0.6 mM isopropyl-β-d-thiogalactopyranoside. Proteins were purified using GST Purification Modules (Amersham Biosciences) according to the manufacturer’s instructions. Purified ChTL1A was analyzed by SDS–PAGE with Coomassie brilliant blue staining. Protein concentration was determined by the Bio-Rad Protein Assay. Antiserum was produced from rabbits by emulsifying ChTL1A in CFA, intracutaneous injection plus six booster inoculations at 2-wk intervals. Blood was collected 10 days after the final inoculation and Ab were purified by protein A-Sepharose affinity chromatography (see below).
**Table I. Primer set for quantitative RT-PCR**

<table>
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<tr>
<th>Gene Name</th>
<th>Primer Set</th>
<th>PCR Product Size (bp)</th>
<th>Accession No.</th>
</tr>
</thead>
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<tr>
<td><strong>IL-1β</strong></td>
<td>5′-ATG GCG TTT GTG GCT GAT GCTG TGG CTG CTG-3′</td>
<td>795</td>
<td>Y15006</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>5′-ACT TAG GCT GAC AGT GAG GTG TGG GTG GAC GGT GCT-3′</td>
<td>238</td>
<td>AJ305540</td>
</tr>
<tr>
<td><strong>IFN-γ</strong></td>
<td>5′-ACT GAG CAT GGG GAG TAG GGT GGT GCT GCT-3′</td>
<td>288</td>
<td>X99774</td>
</tr>
<tr>
<td><strong>ChTL1A</strong></td>
<td>5′-ACT TAC ACA CCC ACG CAC CAC CAC-3′</td>
<td>292</td>
<td>AB194710</td>
</tr>
<tr>
<td><strong>iNOS</strong></td>
<td>5′-CTT AAT GGT CAA GAA GAA GGC TTT-3′</td>
<td>826</td>
<td>U46504</td>
</tr>
<tr>
<td><strong>COX-2</strong></td>
<td>5′-AAG AAC ATA GAG GTG TGG TGC TCT CCA GGA GAG-3′</td>
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<td>M64990</td>
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<tr>
<td><strong>GAPDH</strong></td>
<td>5′-TGT AGG CGT TTT ATC ATC TCA GCT CC-3′</td>
<td>543</td>
<td>AF047874</td>
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</tbody>
</table>

**Overexpression of chicken TNFRs in CHO-K1 cells and cytotoxic assay of recombinant ChTL1A**

The full-length ORF of each chicken TNF receptor was amplified by PCR using the following primer set: chicken TNFα-forward primer 5′-CTC GAG ATG GCG GGC TGG GTG CTG C-3′, reverse primer 5′-GAA TTC AAC AGT TTT CAT CCC CAT ATC T-3′, and plasmid DNA was extracted using Qiagen Prep Miniprep (Qiagen). CHO-K1 cells (Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University, Aoba-ku, Japan) were routinely grown in RPMI 1640 (Invitrogen) containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO2. Cells cultured to subconfluence were washed with PBS. Liposomes of 10 μg/ml plasmid DNA and 4 μg/ml Lipofectamine 2000 (Invitrogen) were added to the cells, which were then incubated for 24 h at 37°C in 5% CO2. The cells were selected by G418 (0.4 mg/ml) and TNF receptor expression was checked by Western blot using HRP-labeled anti-V5 (Invitrogen). Unmatured pIND/V5-His vector was used as a negative control.

To measure the biological activities of ChTL1A on TNFRs, the standard TNF-α bioassay was performed (10). Cells overexpressing each type of receptor were seeded in triplicate in flat-bottom 96-well microplates (2 er Shrem Biosciences) at 4°C overnight. After centrifugation, the suspension was incubated with gentle agitation at 4°C for 4 h. After centrifugation at 500 × g for 5 min, the supernatant was removed, and the Sepharose beads were washed three times with 10-bed volumes of PBS. One hundred fifty microliters of lysate containing TNFR2/FLAG or DcR3/FLAG was added to the Sepharose. The suspension was incubated with gentle agitation at 4°C for 4 h. After centrifugation at 500 × g for 5 min, the supernatant was removed, and the Sepharose beads were washed three times with 10-bed volumes of PBS. One hundred fifty microliters of

**Time course of appearance of ChTL1A in blood following LPS stimulation**

Chickens were injected with LPS (1.5 mg/kg body weight) and venous blood was sampled 0-24 h later. Blood was centrifuged (1500 × g, 10 min) to prepare plasma and this was stored at −20°C. Five hundred microliters of plasma was diluted 1/3 by PBS and 30 μl of anti-ChTL1A serum was added. To immunoprecipitate ChTL1A, anti-ChTL1A IgG was added and the solution was mixed gently at 4°C for 4 h. Following, that a bed volume of 50 μl of protein A-Sepharose CL-4B (Amersham Biosciences) was added and mixing was continued overnight. After the wash, the bound protein was eluted by 40 μl of 1× electrophoresis sample buffer. Electrophoresis, transfer to membrane, and blocking were conducted as described above. The membrane was incubated as above with rabbit anti-ChTL1A Ab (1/200 dilution in PBS-T), washed, and incubated as above with anti-FLAG M2 IgG conjugated to HRP (1/5000 dilution in PBS-T). Immunoblots were visualized on Hyperfilm (Amersham Biosciences) with ECL Western Blotting Substrate (Pierce). The lysate containing TNFR2/FLAG or DcR3/FLAG was used as a positive control.

**Real-time quantitative RT-PCR**

To study expression of factors related to inflammation (IL-1β, IL-6, ChTL1A, IFN-γ, iNOS, and COX-2), four chickens were administered recombinant ChTL1A protein (200 μg/kg body weight). Total RNA was prepared by TRIzol reagent (Invitrogen) from liver, spleen, and abdominal adipose tissue. First-strand cDNA was synthesized as previously described (7). Real-time quantitative RT-PCR analysis was performed using the Cycler iQ Real Time Detection System (Bio-Rad). Each cDNA preparation served as a template in a 50-μl PCR containing 2 mM MgCl2, 0.5 mM of each primer, and 0.5× SYBR Green master mix (BioWhittaker). Cycling conditions were 94°C for 3 min, followed by 35 cycles of 94°C for...
30 s, 60–65°C for 1 min, and 72°C for 1 min. SYBR Green fluorescence was detected at the end of each cycle. At the end of each run, melting curve profiles were also recorded. Sense and antisense primers are shown in Table I.

The specificity of amplification was further verified by electrophoresis of product on a 0.8% agarose gel, to check size, followed by DNA sequencing. Results are expressed as the ratio of each inflammatory factor to GAPDH.

Changes in plasma ceruloplasmin (Cer) and α1 acid glycoprotein (α1 AG) concentration following injection of recombinant ChTL1A

To investigate inflammatory activity of recombinant ChTL1A, changes in Cer and α1 AG were determined following injection of ChTL1A in male 3-wk-old broiler chickens, weighing ~1 kg. Recombinant ChTL1A was dissolved in sterilized PBS. ChTL1A (0.5 ml, 200 μg/kg body weight) and GST (200 μg/kg body weight) were injected i.v. into four birds each. Venous blood was sampled and plasma prepared as described above. Cer concentration was measured by the p-phenylenediamine colorimetric method (11). Plasma (0.1 ml) was diluted in 2 ml of 0.1 M acetic acid buffer (pH 6.4) and incubated at 37°C for 5 min. p-Phenylenediamine (27 mM) was added to the mixture and incubated at 37°C for 30 min. The reaction was stopped by addition of 50 μl of 1.5 M sodium azide and A530 was measured using a 0-min incubation as a blank. Concentration was calculated as: Cer concentration (mg/L) = 752×A530.

Plasma α1 AG concentration was measured by using an ECOS check chicken α1 AG quantitative assay kit (Laboratory of Metabolic Eco System) based on the method of single radial immunodiffusion.

Effect of anti-ChTL1A antiserum on LPS-injected chickens

Fifteen male broiler chicks (Ross), 13 days old, were randomly assigned to one of three experimental groups. Chicks were housed in a temperature-controlled room (25°C) and fed ad libitum on a commercial diet. In each group, the mean bird weight was 480 g.

Five chicks were injected i.v. with protein A-purified rabbit anti-ChTL1A IgG and five with polyclonal rabbit IgG (0.5 mg/kg body weight). After 30 min, all 10 birds were injected i.p. with LPS (1.5 mg/kg body weight) and, after another hour, were injected i.v. with the appropriate IgG as above. Another five chicks received only the LPS injection. Feed intake was recorded every 2 h until 16 h after treatment. Also, to determine nitrite plus nitrate, Cer and α1 AG, blood was taken from a wing vein before treatment and then after 8 and 24 h.

Statistical analysis

We used Student’s t test to compare means. The threshold of significance was 0.05.

![FIGURE 1. Changes in viability of CHO-K1 cells that overexpress full-length chicken TNFRs (TNFR2 or DcR3) following addition of ChTL1A. pIND vector was used as negative control. The effect was quantified by measuring absorbance (OD A = main wavelength: 450 nm; reference wavelength: 655 nm) using a cell counting kit 8. The cell viability was calculated at each ChTL1A concentration/ChTL1A-free viability. **, p < 0.01 compared with values of the pIND vector transfection.](image1)

![FIGURE 2. ChTL1A interacts with chicken TNFR2 (A) and chicken DcR3 (B). ChTL1A/GST or GST was purified by glutathione-Sepharose, and binding to FLAG/TNF receptors was studied by pull-down assay and Western blotting. The lysate containing TNFR2/FLAG or DcR3/FLAG was used as positive control.](image2)

![FIGURE 3. Changes in plasma ChTL1A protein following i.p. injection of LPS (1.5 mg/kg body weight). The protein immunoprecipitated by rabbit anti-ChTL1A Ab was analyzed by Western blot. ChTL1A was detected with rabbit anti-ChTL1A serum used as first Ab and HRP-labeled anti-rabbit IgG Ab and visualized by ECL.](image3)

![FIGURE 4. mRNA expression of IL-1β, IL-6, ChTL1A, IFN-γ, iNOS, and COX-2 in various tissues following i.v. injection of ChTL1A. ChTL1A (0.5 ml; 200 μg/kg body weight, ■) or GST (200 μg/kg body weight, □) was i.v. injected and each tissue was taken after 2 h. GST was used as a negative control. Each bar represents the mean and SE from four chickens. Arbitrary units (AU) are the ratio of each mRNA expression to the GAPDH mRNA expression. Expression of mRNA was measured by real-time PCR, ***, p < 0.01 and *, p < 0.05 compared with control.](image4)
ChTL1A interacts with TNFRs

Expression constructs containing chicken TNFR2 or chicken DcR3 cDNAs, including an additional C-terminal His tag sequence (or pNDV/5-His only as a control), were transfected into CHO-K1 cells. Cells overexpressing chicken TNFR2 or chicken DcR3 demonstrated high sensitivity to ChTL1A in a dose-dependent manner, whereas cells transfected with control pNDV showed low sensitivity (Fig. 1). GST pull-down assays confirmed the interaction between ChTL1A and chicken TNFRs TNFR2 and DcR3 (Fig. 2). These results suggest that ChTL1A signaling is transduced by interaction with either TNFR2 or DcR3.

Responsiveness to LPS of plasma ChTL1A protein levels

We have previously reported the sequential changes in splenic ChTL1A mRNA expression following LPS injection, with peak expression after 2 h (7). In plasma, ChTL1A protein was detected from 2 h after LPS injection, became maximal 4 h after injection, and decreased gradually thereafter (Fig. 3).

Proinflammatory effect of ChTL1A recombinant protein in vivo

FIGURE 5. Changes in plasma concentration of Cer (A) and α1 AG (B) following i.v. injection of ChTL1A. ChTL1A (0.5 ml; 200 μg/kg body weight, ■) or GST (200 μg/kg body weight, negative control, ◦) was injected i.v. The graph (B) shows the increase relative to time 0. Each point represents the mean and SE from four chickens. **, p < 0.01 and *, p < 0.05 compared with value of GST injection at the same time.

Ethics approval

The Animal Care and Use Committee of the Graduate School of Agriculture of Tohoku University approved all procedures.

Results

FIGURE 6. Changes in plasma concentration of Cer, α1 AG, and nitrite plus nitrate, in LPS-treated chickens, 30 min after i.v. injection of rabbit anti-ChTL1A IgG (0.5 mg/kg body weight, ◦) or rabbit IgG (0.5 mg/kg body weight, ⊗). Chickens were injected i.p. with LPS (1.5 mg/kg body weight). One hour after LPS injection, the chicks were injected with each IgG (0.5 mg/kg body weight) i.v. Controls were injected with LPS only (○). Each treatment represents the mean and SD from five chickens. **, p < 0.01 and *, p < 0.05 compared with the value of LPS injection at the same time.

In this study, ChTL1A could bind to both chicken TNFR2 and chicken DcR3 (Fig. 2). A single band was detected by anti-FLAG Ab although there was any weakly signal at other molecular mass tags, suggesting no pull-down protein was detected by anti-FLAG Ab although there was any weakly signal at other molecular mass tags. This implies that ChTL1A is a proinflammatory cytokine in chickens.

Effect of anti-ChTL1A antiserum on LPS-injected chickens

The injection of anti-ChTL1A IgG (total 1.0 mg/kg body weight) blocked the LPS-induced changes in plasma Cer at 24 h, α1 AG at 24 h, and nitrite plus nitrate at 8 h. The Cer, α1 AG, and nitrite plus nitrate plasma concentrations in chickens injected with rabbit IgG (control) did not differ from those seen after LPS injection (13–15). ChTL1A fusion protein and GST preparations did not contain detectable levels of LPS (<100 ng/mg protein) using an Endospeck ES-50M kit (Seikagaku). Since the recombinant protein was injected at 200 μg/kg body weight, LPS contamination was presumably <20 ng/kg body weight. There were no significant differences in plasma inflammatory parameters between an i.v. LPS-injected control group (50 ng/kg body weight) and a PBS-injected group (data not shown). This implies that ChTL1A is a proinflammatory cytokine in chickens.

Discussion

Binding with receptor and signal transduction

In mammals, TL1A is reported to bind to DcR3 and DR3 (16) and its signals are transmitted through DR3, but not DcR3 (9). It is well known that TNF-α binds to two receptors, TNFR1 and TNFR2, and can result in apoptosis (1, 17). In birds, TNFR2 and DcR3 cDNA have been reported (18, 19), but the TNFR1 and DR3 homologs are yet to be identified. We therefore investigated whether ChTL1A binds to chicken TNFRs, TNFR2 or DcR3.

In mammals, TL1A is reported to bind to DcR3 and DR3 (16) and its signals are transmitted through DR3, but not DcR3 (9). It is well known that TNF-α binds to two receptors, TNFR1 and TNFR2, and can result in apoptosis (1, 17). In birds, TNFR2 and DcR3 cDNA have been reported (18, 19), but the TNFR1 and DR3 homologs are yet to be identified. We therefore investigated whether ChTL1A binds to chicken TNFRs, TNFR2 or DcR3.

In this study, ChTL1A could bind to both chicken TNFR2 and chicken DcR3 (Fig. 2). A single band was detected by anti-FLAG Ab although there was any weakly signal at other molecular mass in the DcR3 experiment. In addition, the GST pull-down assay was also used with empty vector as a negative control, and mixed with the receptor fusion proteins, no pull-down protein was detected by Western blot. Thus, our data of GST pull-down assays shows the
specific binding of ChTL1A to these receptors. In addition, recombinant ChTL1A has marked cytotoxic activity against cells over-expressing chicken TNFRs TNFR2 and DcR3. Thus, we have demonstrated for the first time that in birds, the toxic activity of ChTL1A involves chicken TNFR2 and chicken DcR3. This suggests that the mechanisms that induce cell death in chickens and mammals are different. In mammals, DcR3 is secreted into the blood, binds with the ligand for DR3 and, consequently, inhibits DR3 signal transduction (20). In contrast, our results demonstrated that the CHO-K1 cells transfected with chicken DcR3 still had high cytotoxic sensitivity induced by ChTL1A in a dose-dependent manner, whereas cells transfected with control pIND only showed very low cytotoxic sensitivity (Fig. 1). This suggests that DcR3 might induce cell death in chickens, but further investigation is needed. A predicted sequence for TNFR1 can be found in the chicken genome database (21); however, the connection between ChTL1A and these receptors remains to be elucidated. These receptors were expressed in different tissues in chicken. TNFR2 was reported to be mainly expressed in spleen and bursa of Fabricius, but little expressed in liver (18). In contrast, DcR3 was reported to be mainly expressed in oviduct but little expressed in spleen (19). Our study showed that the response to ChTL1A in the spleen was higher than in other tissues (Fig. 4). Therefore in spleen, ChTL1A might interact with TNFR2 rather than DcR3 and promote inflammation. In vivo injection of chicken TNFR2 and/or DcR3 Abs may elucidate the proinflammatory mechanism.

ChTL1A protein secretion into plasma during inflammation

We previously reported that in chicken spleens, ChTL1A mRNA increased following LPS injection, with peak expression 2 h after injection (7). In the present study, proteins of ~17 kDa were detected in plasma 4 h after LPS injection (Fig. 3). In mammals, TNF-α binds to membranes as a 26-kDa precursor protein, where it is cleaved sequentially by TNF-α-converting enzyme (TACE), releasing into the blood the 17-kDa form (22). Human TL1A is also a secretion protein that has a similar transmembrane domain to TNF-α (9). Our previous study predicted that ChTL1A might have transmembrane domain and TACE cleavage site (7). It is likely, therefore, that ChTL1A is cleaved by TACE in a similar manner to TNF-α in mammals because TACE has been identified in the chicken genome (21). Thus, in chickens, ChTL1A can be secreted into plasma, suggesting it may both play a local autocrine and/or paracrine role and also be circulated. In humans, plasma TNF-α concentrations peak 2–4 h after LPS injection (23). Thus, our findings in chickens are similar to those in mammals. In addition, a 17-kDa protein was detected using anti-human TNF-α Ab in chicken macrophage culture medium (6). Taken together, the current results suggest that ChTL1A is a secreted molecule with TNF-α-like activity.

The importance of ChTL1A in inflammation

In the first stage of infection, an inflammatory factor is expressed that subsequently influences both the immune and metabolic responses. In mammals, TNF-α promotes IL-1β and IL-6 production, i.e., it is a significant inflammatory cytokine (3, 24). This similarity to ChTL1A was confirmed when increased expression of IL-1β and IL-6 was observed following ChTL1A injection (Fig. 4). ChTL1A injection also increased the expression of IFN-γ and ChTL1A itself. ChTL1A, therefore, induces inflammation through the same cytokines.

Because ChTL1A has similar proinflammatory actions to mammalian TNF-α (Fig. 5), we wondered whether it might also activate NF-κB signaling through TNFR2. Unfortunately, we were unable to test this because the function of chicken TNFR2 has not been completed to date. ChTL1A injection also increased the expression of iNOS and COX-2. The effect of ChTL1A to increase plasma NO concentration confirmed the results of our previous study (7) and could be due to an increase in iNOS expression by the liver and spleen.

In mammals, TNF-α is an important proinflammatory cytokine that regulates a host’s response to infection as well as inflammation and trauma. Acute phase protein production is induced mainly by proinflammatory cytokines such as IL-1, IL-6, and/or TNF-α, while NO production is induced by IL-1, TNF-α, and/or IFN-γ. Thus, proinflammatory cytokines are also important regulators of metabolic responses in the acute phase of inflammation. In chickens, α1 AG, Cer, and NO are acute phase substances released following LPS injection (13, 14, 25). Our results demonstrate that ChTL1A promotes inflammation by raising these. For TNF-α, this effect results from IL-6 induction (26). In our study, the peak of acute phase protein occurs after the peak of NO production; hence ChTL1A may indirectly induce acute phase reactants through IL-6 induction rather than directly as mammalian TNF-α functions. Therefore, we suggest that, in chickens, the mode of action of ChTL1A is similar to that of TNF-α in mammals in acute phase protein induction.

Finally, we observed that anti-ChTL1A IgG down-regulated inflammation. We previously ascertained, that following LPS injection under similar conditions, plasma nitrate plus nitrite become maximal after 9–14 h, and plasma Cer and α1 AG become maximal after 24 h (13–15). We therefore measured the concentration of Cer at 24 h, α1 AG, at 24 h and nitrite plus nitrate at 8 h. This suggests that ChTL1A is important in the inflammatory response. ChTL1A plays a significant role in the initiation of inflammation, because its Ab suppressed NO production and suppressed acute phase protein production. The results show that ChTL1A is an important regulating factor for both immune and inflammation responses in chickens and may do the same job as mammalian TNF-α functions in chickens, animals that lack genes orthologous to TNF-α and lymphotixin-α.

In conclusion, we demonstrated that ChTL1A binds to TNFR2, the receptor for TNF-α in mammals, and its signals are transmitted via TNFR2. ChTL1A proteins were detected in plasma 4 h after LPS injection, suggesting that ChTL1A is secreted into the blood, like mammalian TNF-α, and subsequently influences the immune responses of other tissues. ChTL1A plays an important role as a proinflammatory cytokine, as recombinant ChTL1A increased the expression of several inflammation-related factors such as iNOS and COX-2 in chicken spleen. In plasma, it increased the concentrations of acute phase proteins. Anti-ChTL1A IgG abolished the increase in the acute phase reactants following LPS injection.

These results indicate that ChTL1A is a key proinflammatory cytokine in chickens, animals that do not have TNF-α and lymphotixin α orthologous genes, and that the proinflammatory actions of ChTL1A occur, at least in part, through binding to TNFR2.

Disclosures

The authors have no financial conflict of interest.

References


