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Characterization of the chicken interleukin-1β converting enzyme (caspase-1) cDNA and expression of caspase-1 mRNA in the hen

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Abstract

We have cloned and sequenced a chicken homolog to the mammalian interleukin-1β (IL-1β)-converting enzyme (caspase-1) cDNA and have evaluated caspase-1 mRNA expression in various tissues from the domestic hen, including ovarian follicle granulosa and theca layers. The deduced amino acid (aa) sequence of chicken caspase-1 is 44.9% identical to human caspase-1, and contains an active site pentapeptide that is characteristic of the caspase family of cysteine proteases. Of interest, however, is that the putative chicken caspase-1 cDNA is predicted to encode a comparatively short (19 aa) N-terminal prodomain, as well as two Cys residues within the active pentapeptide (QC^{162}C^{163}RG) as compared to the QACRG pentapeptide found in the mammalian caspase-1 sequence. While the chicken caspase-1 mRNA transcript is widely expressed among different tissues, levels are particularly high in the bursa of Fabricius and comparatively low in ovarian follicles at all stages of development. Finally, treatment of granulosa cells with IL-1β, the primary if not sole product of caspase-1 activity, fails to either promote apoptotic cell death or enhance viability in granulosa cells. Considering the relatively low levels of caspase-1 mRNA expression in ovarian follicle tissues plus the inability of IL-1β to alter granulosa cell viability, in vitro, it is concluded that caspase-1 is not an integral part of the apoptotic pathway in granulosa cells. However, the pattern of mRNA expression is consistent with a requirement for caspase-1 mediated IL-1β production in chicken immune tissues. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Interleukin-1β; *ced*-3; Apoptosis; Ovarian follicle; Granulosa; Theca

1. Introduction

Caspase-1 (interleukin-1β-converting enzyme) represents a cysteine protease that cleaves pro-interleukin-1β following an aspartic acid residue to generate the mature cytokine, interleukin-1β (IL-1β). The primary, if not sole, product of this protease is IL-1β, although there is some recent evidence implicating caspase-2 activity in the proteolytic maturation of interferon-γ inducing factor within T-cells (Wilson et al., 1994; Gu et al., 1997). The nt sequence of human caspase-1 is homologous to *ced*-3, a gene required for mediating apoptosis during development in *Caenorhabditis elegans* (Yuan et al., 1993). In mammalian species, active caspase-1 consists of two subunits (p20 and p10) that are derived from a 45-kDa precursor protein following auto- or heterocatalysis (Kumar, 1995). The active site of mammalian caspase-1 resides in the p20 subunit and consists of the pentapeptide, Gln–Ala–Cys–Arg–Gly (QACRG). Subsequent studies in the human have demonstrated the existence of at least 10 additional homologous proteases, caspase-2 through -11 (Alnemri et al., 1996; Miller, 1997; Nicholson and Thornberry, 1997), that together constitute the caspase family of cystein proteases. All mammalian caspases identified to date contain a conserved QACXG pentapeptide, where X represents an Arg (R), Gln (Q) or Gly (G). Within this family of enzymes, caspase-4 (Ich-2 or ICE_{rel-II}; Kamens et al., 1995; Munday et al., 1995) and caspase-5 ($ICE_{rel-III}$; Munday et al., 1995) are considered to be most homologous to caspase-1, and together they represent the caspase-1 subfamily (Alnemri et al., 1996). Members of the caspase-1 subfamily are thought to predominantly play a role in inflammation. By comparison, two addi-

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Abbreviations: aa, amino acid(s); bp, base pair(s); caspase-1, Interleukin-1β-converting enzyme; caspase-2, Interleukin-1β-converting enzyme and Ced-3 homolog, or Ich-1; cDNA, complementary DNA; IL-1β, interleukin-1β; kb, kilobase or 1000 bp; mRNA, messenger RNA; nt, nucleotide; ORF, open reading frame; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA end(s).

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tional subfamilies, consisting of a Ced-3 subfamily (caspases-3, -6, -7, -8 and -10) and an Ich-1 subfamily (caspases-2 and -9) are proposed to be largely linked to mediating apoptosis. To date, identification and characterization of only a single avian caspase, chicken caspase-2 (Ich-1; Wang et al., 1994; Johnson et al., 1997a), has been reported. This is despite biochemical evidence for at least five avian caspase-like proteases each with distinctive substrate recognition properties (Takahashi et al., 1997).

Several lines of evidence indicate that caspases, including caspase-1, are directly involved in mediating apoptosis via the cleavage of intracellular proteins (Kumar, 1995; Cohen, 1997). For instance, over-expression of caspase-1 in a fibroblast (Rat-1) cell line induces apoptosis, and this activity can be blocked by co-expression of Bcl-2, an inhibitor of apoptotic cell death, and CrmA, a cowpox virus protein that is a selective inhibitor of caspase activity (Miura et al., 1993). However, there is also evidence that caspase-1 itself does not have a critical, non-redundant role in mediating apoptosis in most tissues, as mice in which the caspase-1 gene has been specifically deleted develop normally and are apparently healthy and fertile (Kuida et al., 1995; Li et al., 1995).

It is now well established that ovarian follicle atresia in mammals and the domestic hen is mediated via apoptosis, and that such apoptotic cell death occurs primarily, if not exclusively, within the granulosa cell layer (e.g. Johnson et al., 1996; Johnson, 1997). A unique aspect of the hen ovarian follicle hierarchy is that susceptibility to apoptosis varies with the stage of follicle differentiation (Johnson et al., 1996). Granulosa cells from prehierarchical (6–8-mm-diameter) follicles are highly susceptible to apoptosis when incubated as a suspension culture for 3–6 h. By comparison, granulosa cells from the largest preovulatory (F1) follicle are highly resistant to apoptosis when cultured in vitro. Previous reports have suggested that IL-1β may play a regulatory role in the process of ovarian follicle atresia via its ability to attenuate apoptosis (Chun et al., 1995). Thus, the objectives of the present study were to characterize the chicken caspase-1 cDNA, evaluate the expression of caspase-1 mRNA in hen tissues, and investigate the ability of mature IL-1β to affect the viability of apoptosis-susceptible and -resistant granulosa cells, in vitro.

2. Materials and methods

2.1. Animals

Single-comb white Leghorn hens (H&H Poultry, Portland, IN), 25–40 weeks of age and laying regular sequences of at least eight to 10 eggs, were used in all studies described. Birds were housed individually in laying batteries, provided with free access to feed (Purina Layena Mash, Purina Mills, St. Louis, MO) and water, and were exposed to a photoperiod of 15 h of light, 9 h of darkness, with lights on at 00.00. Individual laying cycles were monitored by the daily timing of oviposition. Hens were killed approximately 16–18 h prior to a midsequence ovulation by cervical dislocation. All procedures described herein were reviewed and approved by the University of Notre Dame Institutional Animal Care and Use Committee, and were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals.

2.2. Isolation and characterization of chicken caspase-1 cDNA, and deduced aa sequence

Degenerate primers, based upon highly conserved QAC(R/G/Q)G and GS(V/W)(F/Y)I pentapeptides found within mammalian caspases, were used to initially amplify a 305-bp chicken caspase-1 cDNA homolog from reverse-transcribed mRNA collected from chicken thymus tissue [forward primer = CAGGC(T/C)TG-CCG(T/A/G)GG; reverse primer = GAT(G/A)(A/T)-ACCAGGA(G/T)CC]. The nt sequence of this product was utilized for the design of additional specific primers to amplify the 5′ and 3′ ends of chicken caspase-1 by RACE (Clontech, Palo Alto, CA) using Poly(A)$^+$-enriched (Micro-Fast Track kit; Invitrogen, San Diego, CA) reverse-transcribed RNA obtained from granulosa tissue of the second largest (F2) preovulatory plus prehierarchical (6–8-mm-diameter) follicles. Double-stranded cDNA was synthesized, and Marathon cDNA Amplification adaptors (Clontech) were ligated. Two different sets of nested gene-specific primers were designed from the nt sequence obtained from the chicken caspase-1 PCR product for the 5′ direction [GSP1: 5′-CCAGGAGACAGTATCAGGCGTG-3′ (bp 1330–1309) combined with GSP2: 5′-ATCCGAAGTGG-AAGTGGGCATC-3′ (bp 1228–1207); and GSP5: 5′-CTTGCCCTGCTCATGGACTACAG-3′ (bp 681–659) combined with GSP6: 5′-CCATGCTCCA-GCCCTTCGCTC-3′ (bp 615–595)]. One set of nested primers was utilized for the 3′ direction [GSP3: 5′-ACCAGAGAAGCTCACCTGGAGAG-3′ (bp 1262–1284); and GSP4: 5′-CCGTTTTTATCCAGAGACT-GGTGG-3′ (bp 1350–1373)]. An initial amplification of the 5′-region by PCR was performed using GSP1 and Adaptor Primer 1 (Clontech) under the following conditions: 1 min, 94°C; 0.5 min, 94°C and 4 min, 72°C for five cycles; 0.5 min, 94°C and 4 min, 70°C for five cycles; 0.5 min, 94°C and 4 min, 68°C for 25 cycles. A second amplification was conducted with the GSP2 and Adaptor Primer 2 as internal primers and amplification conditions as described above. This procedure was repeated using the second nested pair of primers. PCR

products were gel-purified and ligated into the PCR2.1 vector (TA Cloning Kit, Invitrogen), then further amplified and purified from plasmid preparations. Amplified and cloned chicken caspase-1 nt sequence was determined by nucleic acid sequencing using the dideoxy chain termination method (Sanger et al., 1977) and the Sequenase version 2.0 system (Amersham, Arlington Hts, IL). Initial and secondary amplifications of the 3′-region were conducted utilizing GSP3 plus Adaptor Primer 1 and GSP4 plus Adaptor Primer 2, respectively, with the same amplification conditions described above. PCR products were gel-purified and ligated into the PCR2.1 vector, amplified and purified from plasmid preparations. Sequence data for caspase-1 were assembled from replicate products over the entire coding region and analyzed using the MacVector and AssemblyLIGN programs (version 4.5.3; IBI, New Haven, CT). The consensus sequence shown represents information from at least two different clones sequenced in both directions. Furthermore, a total of four different clones were sequenced from the ATG start codon through the start of the 5′ region, while reverse-transcribed RNA from two different hens was used to obtain the sequence through the QCCRG catalytic region.

2.3. Northern blot analysis

The total cellular RNA from each tissue and stage of follicle development was isolated using Trizol Reagent (Gibco-BRL, Gaithersburg, MD), while the purity and quantity were assessed by measuring the optical density of each sample at 260 nm versus 280 nm. Fifteen micrograms of total cellular RNA were electrophoresed on 1% agarose gels in the presence of formaldehyde, then transferred to nitrocellulose Nitro ME membranes (MSI, Westboro, MA) by capillary action overnight. Blots were incubated with a chicken caspase-1 ^{32}P-labelled cDNA probe (corresponding to nt 1148–1360) at 60°C overnight and subsequently washed under highly stringent conditions at 60°C. When appropriate, blots were rehybridized to a random-primed chicken 18S ribosomal RNA cDNA probe to standardize for equal loading of RNA samples (Johnson et al., 1997b). the extent of hybridization was quantitated using a phosphoimager equipped with the ImageQuant data reduction system (Molecular Dynamics, Sunnyvale, CA). Data are expressed as the fold-difference compared to the 3–5-mm-diameter follicle ± SEM, and represent three or four replicate experiments.

2.4. Granulosa cell incubation and culture experiments

Granulosa cells from 6–8-mm follicles were incubated in 12 × 75-mm polypropylene tubes at 37°C in medium M199 plus HEPES (Johnson et al., 1997b) for 0–6 h in the absence or presence of 1, 10 and 33 ng of murine recombinant IL-1β/ml (Gibco-BRL). F1 follicle granulosa cells were plated in six-well Falcon culture plates (Becton Dickinson, Lincoln Park, NJ) for 24 h in the absence or presence of 1 and 10 ng of IL-1β/ml. Following incubation or culture, genomic DNA was prepared from cells, and 1 μg was electrophoresed on a 1.8% agarose gel in the presence of SYBR Green I (1:20 000 dilution in the agarose gel; Molecular Probes, Eugene, OR). The extent of oligonucleosome formation was determined by quantitating fluorescence of the low-molecular-weight (< 20 kb) DNA bands (Johnson et al., 1996) using the phosphoimaging system equipped with the ImageQuant data reduction system. Data represent the mean fold-difference in oligonucleosome formation ± SE compared to the appropriate control from three replicate experiments.

3. Results and discussion

3.1. Cloning of a cDNA encoding the chicken homolog of mammalian caspase-1

The total length of the cDNA characterized is 1765 nt, including a predicted 849-bp open reading frame (Fig. 1). The homology of the chicken versus human caspase-1 nucleic acid sequences within the predicted open reading frame is 60.8%. The deduced aa sequence from the chicken caspase-1 cDNA shows 44.9% identity (62.5% similarity) to human caspase-1 (Fig. 2). The remaining two members of the human caspase-1 subfamily, caspase-4 and caspase-5, share 41.7 and 39.6% identity, respectively, to chicken caspase-1. By comparison, there was considerably less homology to the only other chicken caspase thus far identified, caspase-2 (24.0% aa identity); this degree of identity is comparable to that which is found between the human caspase-1 and -2 proteins (∼22%) (Cohen, 1997).

Of note was that the putative start site for the chicken caspase-1 corresponded to Met^{123} from the human caspase-1 aa sequence. It has previously been documented that the greatest degree of variability among the human caspase homologs occurs within the N-terminal prodomain. For instance, caspase-9 has one of the longest N-terminal prodomains (130 aa), whereas caspase-6 and -7 have the shortest (23 aa; Fernandes-Alnemri et al., 1995a,b). Interestingly, the putative start codon for chicken caspase-1 most closely corresponds with that of the human caspase-6 aa sequence, and the projected prodomain for chicken caspase-1 terminates with Asp^{19}. Otherwise, however, human caspase-6 and chicken caspase-1 share only 24.6% aa identity (46.7% similarity). Importantly, the site of initial cleavage that results in the formation of active fragments, Asp^{175}–Ser^{176}, is conserved between the chicken and mammalian caspase-1.

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TGACA ACCGA CAAGG CCCGC AGCCT CATAG ACACC GTACG CCTGA AGGGC CCTCG GCCAG    60

CGCCA TCTTC ATTGA CAGCC TCAGG AAGCA CGACT GCAAC TTGGC AGAGC AGCTG AGACT   120

GTGTG CTCAT GCAGG TGAGA GGCAG GGCCA TTGGC CTGCC CCGGC CATGC TTGGG CTCTC   180

AGTGC CTCTC ATGGC GTGCT GGAGA GAGGG TCAGC CTTGC CCTCT GGCCC AGGCC CAAAG   240

ACACT GAGCA GTCAG GAATT CCCAG TGTCA CTGTG CTGTG GCAGT GTGTA CCCCT CACCA   300

CGTGG TGACA ACAGG GCTCT CAGGT GGCTG TCCCC TGTAG GCATC CACTG TGGGG CAGAG   360

ATGGG CTCTG CTTGC CCCCC ACGCA CTGTG ACAGC TCCCT CTTGC CCTTT CCAGT GTAAC   420

CACCT TTGTG CTTGC AGAAC CTCCT GGTGC ACCGC TGGCT GCCCC CAGCA CTGTG GGATT   480

CTCCG ACCCC CCTGT TAGCA GCCAG GACCT GCAGT GGATA CAGCA GTGCC CCCTC AGCGA   540

GTACC AGCGC ATCAA GGAGG CAGAG GGAGA TCAGG TGCTG TGGGT AGAGG AGATG AGCGA   600

AGGGC TGGAG CATGG AGCAG CCTCT CTGCC AGAGG GGGCA AAGGC TGCCA AGGGT CCTCT   660

GTAGTCC ATG AGC AGG GCA AGA TCT TCG GGA AGA TGC CCC AGG GCC AGG GAG ATG   715
        Met Ser Arg Ala Arg Ser Ser Gly Arg Cys Pro Arg Ala Arg Glu Met    16

TGC ATA GAC CTT GTG CAT CAC CTC GTT CTC TCA TCA CAG ATC TAT GAC ATA TAC   769
Cys Ile Asp Leu Val His His Leu Val Leu Ser Ser Gln Ile Tyr Asp Ile Tyr    34

CAG CCA CGG GAG AGA CGA ACC CGT AGG GCC CTG CTC ATC TGC AAC ACC AAG TTT   823
Gln Pro Arg Glu Arg Arg Thr Arg Arg Ala Leu Leu Ile Cys Asn Thr Lys Phe    52

AAG CAC TTG AGA CAG CGG GAC GGA GCT GAA GTG GAC GTG AAG GAG ATG ACA AAG   877
Lys His Leu Arg Gln Arg Asp Gly Ala Glu Val Asp Val Lys Glu Met Thr Lys    70

CTG CTA GAA GGG CTG GGC TAC AAT GTG GAG TGC CAT GAA GAC AAA ACT TCC CAG   931
Leu Leu Glu Gly Leu Gly Tyr Asn Val Glu Cys His Glu Asp Lys Thr Ser Gln    88

GAG ATG ACC ACA GTC ATG AAG AAG TTT GCA GAT CAC AAA GAT CAT TTG ACC TCT   985
Glu Met Thr Thr Val Met Lys Lys Phe Ala Asp His Lys Asp His Leu Thr Ser   106

GAC AGC ACC TTC CTG GTG TTC ATG TCA CAT GGA ATG AGC ACT GGG ATC TGC GGG  1039
Asp Ser Thr Phe Leu Val Phe Met Ser His Gly Met Ser Thr Gly Ile Cys Gly   124

ACC AAG AGT AAT GGG ACC ACG GAC ATC CTT TCC TTC AAC ACC ATC TAC GAG AAT  1093
Thr Lys Ser Asn Gly Thr Thr Asp Ile Leu Ser Phe Asn Thr Ile Tyr Glu Asn   142

TTC AAC AAC AAG CAC TGC AGG GCG CTG ATG GGC AAA CCC AAA GTG GTC ATT ATC  1147
Phe Asn Asn Lys His Cys Arg Ala Leu Met Gly Lys Pro Lys Val Val Ile Ile   160

CAG TGC TGC CGT GGA GAC AAC ATA GGA TCT GTG CAG ATA CGT GAC TCC ATC GAC  1201
Gln Cys Cys Arg Gly Asp Asn Ile Gly Ser Val Gln Ile Arg Asp Ser Ile Asp   178

CCT GAG ATG CCC ACT TCC ACT TCG GAT GGC TGG AGA TGT GTA GAG CTG CAA GGT  1255
Pro Glu Met Pro Thr Ser Thr Ser Asp Gly Trp Arg Cys Val Glu Leu Gln Gly   196

TCT AAA ACC AGA GAA GCT CAC CTG GAG AGT GAT TTT GCC ACT TTA TAT TCT TCC  1309
Ser Lys Thr Arg Glu Ala His Leu Glu Ser Asp Phe Ala Thr Leu Tyr Ser Ser   214

ACG CCT GAT ACT GTC TCC TGG AGA AGC CCC ACA GAA GGT TCC GTT TTT ATC CAG  1363
Thr Pro Asp Thr Val Ser Trp Arg Ser Pro Thr Glu Gly Ser Val Phe Ile Gln   232

AGA CTG GTG GAG CAG TTT CGA AAC CAT GCC TTT AAC AGT GAC TTG CAG GAG ATG  1417
Arg Leu Val Glu Gln Phe Arg Asn His Ala Phe Asn Ser Asp Leu Gln Glu Met   250

TTC CGA AAG GTC CAA CGT TCC TTC GAA AAC TTT CCT CGG CAG TTG CCA ACA CAA  1471
Phe Arg Lys Val Gln Arg Ser Phe Glu Asn Phe Pro Arg Gln Leu Pro Thr Gln   268

GAG AGG ACT ACA ATG CTG AAG AAG TTC TAT CTC TTC CCA GGC CTC TGA T CTGCC  1525
Glu Arg Thr Thr Met Leu Lys Lys Phe Tyr Leu Phe Pro Gly Leu ***           283

CACGC CTCTG AACAA CCTCA AACTG ACCGA ATTCT TGCCA GGGAG CAGCA AAGAT ACTCT  1585

GAACC AAAAC ACTAC GTGCA CACCT GGTGA ACTTC TCTCT ATCAC CCCAA AGAAA GGCTG  1645

TGAAG CAAAC ATCTC CCCAT TCGTG ACAGA TACYT TCCGT CTCTC TGAGA TCCAC TTCTG  1705

CCCTG TTTCT GACAC AACTT GTTGA CTAAT AAAAG GATTA GAACA CGTGA CAGCA AAAAA  1765
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Fig. 1. The nt and deduced aa sequences of chicken caspase-1. The putative ATG start codon and TGA stop codon (***) begin at bp 668 and 1517, respectively. The QCCRG active catalytic site is underlined. The GenBank Accession No. for this nt sequence is AF031351.

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c Caspase-1        MSRARSSGRCPRAREMCIDLVHHLVLSSQIYDIYQPRERRTRRALLICNTKFKHLRQRDG   60
h Caspase-1        .ptssg.egnvklcsleeaqriwkqk.ae..p.md-kss...l..i...ee.dsipr.t.  181
h Caspase-4        ppesge.tdalklcpheeflrlckeraee..p.-ke.nn...l..i....e.d..pp.n.  154
h Caspase-5        aestnilkl...e-.f-lr.ckk-n-hde..p.-kk..d.r.l..i......d..pa.n.  195
c Caspase-2        ehsld.gdgp.ippvkhctpefyrdhqhlaykliseprglalilsn.hfsse.d.ey.s.  192

                                                                          +
c Caspase-1        AEVDVKEMTKLLEGLGYNVECHEDKTSQEMTTVMKKFADHKDHLTSDSTFLVFMSHGMST  120
h Caspase-1        ....itg..m..qn...s.dvkknl.asd...elea..hrpe.k............ire  241
h Caspase-4        .df.itg.ke.....d.s.dve.nl.ard.esalra..trpe.ks...........ile  214
h Caspase-5        .hy.ivg.kr..q....t.vdeknl.ard.es.lra..arpe.ks...........ile  255
c Caspase-2        gd..caslel.fkh...q.tvfh.qsae..esaler.sklp..qdv..civall...veg  252

c Caspase-1       GICGT-KSNGTTDILSFNTIYENFNNKHCRALMGKPKVVIIQCCRGDNIGS-VQIRDSIDPE  180
h Caspase-1       ....k.h.eqvp...ql.a.fnml.t.n.ps.kd....i...a....sp.v-.wfk..vg--  300
h Caspase-4       .....vhdekkp.v.lyd..fqi...rn.ls.kd....i.v.a...a.r.e-lwv...-pas  274
h Caspase-5       .....ahkkkkp.v.lyd..fqi...rn.ls.kd....i.v.a...ekh.e-lwv...-pas  315
c Caspase-2       .vy..---d.--kl.qlqeafrl.d.an.pn.qn...mff..a....etdrg.dq..gkers  309

c Caspase-1        MPTSTSDGWRCVELQGSKTREAHLESDFATLYSSTPDTVSWRSPTEGSVFIQRLVEQFRN   240
h Caspase-1        vsgnl.lp-tte.feddaikk..i.k..iafc.....n....h..m.....g..i.hmqe   359
h Caspase-4        levas.qs--sen.eedavykt.v.k..iafc....hn....ds.m..i..tq.itc.qk   332
h Caspase-5        lavis.qs--sen.eadsvcki.e.k..iafc....hn....dr.r..i..te.itc.qk   373
c Caspase-2        -dspgceesdankeenl.l.lpt-r..micg.aclkg.aam.ntkr..wy.ea.ttv.ae   367

c Caspase-1        HAFNSDLQEMFRKVQRSFEN-FP----------R-QLPTQERTTMLKKFYLFPGL*       283
h Caspase-1        y.csc.ve.i....rf...qpdg----------.a.m..t..v.ltrc......h*       404
h Caspase-4        yswcch.e.v.....q...tpra----------ka.m..i..ls.try......n*       377
h Caspase-5        ysccch.m.i.....k...vpqa----------ka.m..i..a.ltrd......n*       418
c Caspase-2        dsrdthvad.lv..n.qikqr-egyapgtefhrckemseyc-s.lcrdl....yvpgk*    424
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Fig. 2. Alignment of deduced aa sequence form chicken (c) caspase-1 versus the c caspase-2 (Johnson et al., 1997a), and human (h) caspase-1 (Thornberry et al., 1992), caspase-4 and caspase-5 (Munday et al., 1995) proteases. Residues identical to chicken caspase-1 are indicated by '.', and variations from c caspase-1 are in lower-case letters. '−' represents aa absent in the particular sequence. The active catalytic site is designated by bold type, and the conserved His residue marked by '+' has also been implicated in catalysis. Double-underlined regions represent regions that form the Asp pocket in human caspase-1 (Wilson et al., 1994), and are highly conserved within the family of caspases. '*' indicates the predicted stop codon.

It is also noteworthy that the active-site pentapeptide of chicken caspase-1 (QCCRG) differs from the chicken caspase-2 and mammalian caspase sequences by encoding for a Cys substituted for Ala at position 162 (Fig. 2). This substitution was confirmed in multiple cDNA clones from two different hens, and represents a clear deviation from other known caspases. It is unclear what effects, if any, this substitution might have on enzyme activity or substrate specificity. However, conservation of the active site Cys residue, Cys^{163}, appears to be critical, as mutations at this site result in the loss of protease activity (Cohen, 1997).

3.2. *Expression of chicken caspase-1 mRNA in hen tissues*

Caspase-1 mRNA (a single 1.7-kb transcript) was detected by Northern blot analysis in all tissues investigated, though the levels were highest in the bursa of Fabricius, spleen and bone marrow and lowest in the cerebrum, adrenal and ovarian follicle granulosa (Fig. 3). The high mRNA expression levels in the bursa, spleen and bone marrow are proposed to indicate the relative importance of caspase-1 activity and/or IL-1β actions in these critical immune system tissues. For instance, IL-1β is known to modulate immune responses by activating effector cells and inducing production of additional cytokines. Overall, the wide-ranging tissue distribution of chicken caspase-1 mRNA expression and its comparatively high levels in an immune system-related tissue are consistent with results from the mouse (Nett et al., 1992).

The levels of caspase-1 mRNA are comparatively low in both granulosa and theca layers, and were determined not to change significantly ($p > 0.05$) in either tissue during follicle development (Fig. 4). This finding is not unlike the results from the hen ovary reported for chicken caspase-2 mRNA (Johnson et al., 1997a), and is not necessarily unexpected due to the common assumption that the regulation of caspase activity levels is primarily accomplished by the cleavage of the inactive proenzyme to the active form (Cohen, 1997).

3.3. *Inability of IL-1β to alter the progression of apoptosis in hen granulosa cells*

It has previously been determined that granulosa cells from hen ovarian follicles are inherently susceptible or

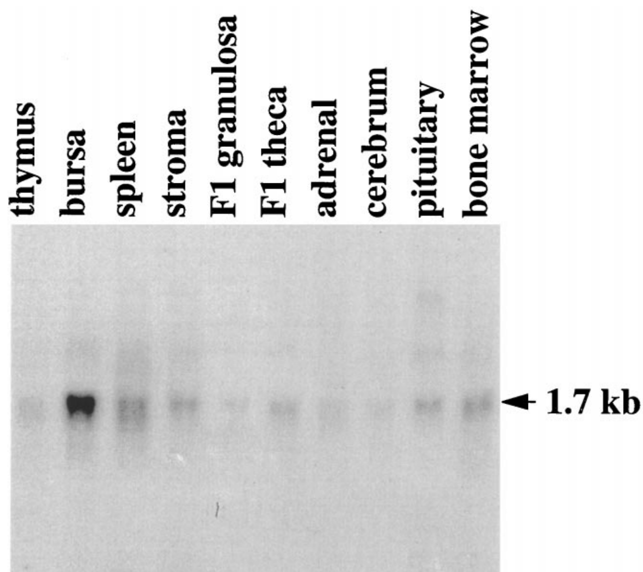

Fig. 3. Representative Northern blot of caspase-1 mRNA in various chicken tissues. Chicken caspase-1 mRNA was detected as a 1.7-kb transcript. F1 refers to the largest preovulatory follicle. Northern analysis was repeated at least twice for all samples shown, with similar results.

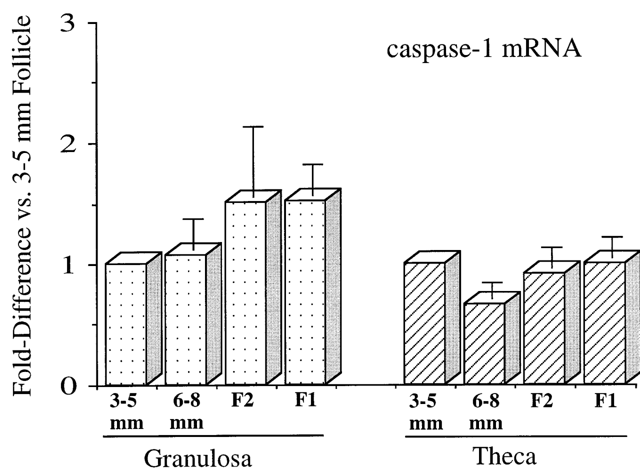

Fig. 4. Summary of densitometric scanning data for caspase-1 mRNA standardized to 18S rRNA following Northern blot analysis of granulosa and theca tissue at various stages of ovarian follicle development. There are no differences among c caspase-1 mRNA levels in either follicle tissue at any stage of development ($p > 0.05$). 3–5 mm and 6–8-mm, prehierarchal follicles; F2, F1, second largest and largest preovulatory follicle, respectively.

resistant to undergoing apoptosis, depending upon the stage of follicle development (Johnson et al., 1996). Specifically, granulosa cells from prehierarchical 6–8-mm follicles are extremely susceptible to undergoing apoptosis within 3–6 h in suspension culture, and the progression of apoptosis can be attenuated by cotreatment with 8-bromo-cAMP (Johnson et al., 1996). By comparison, granulosa cells from preovulatory follicles are virtually resistant to cell death when cultured, in vitro, but apoptosis can be induced by several stimuli, including UV irradiation, ceramide or daunorubicin treatment (Witty et al., 1996). Given previously published results indicating that IL-1β may influence the incidence of mammalian ovarian follicle atresia (Chun et al., 1995), experiments were conducted to evaluate whether treatment of hen granulosa cells with IL-1β, in vitro, would promote oligonucleosome formation (a hallmark of apoptosis) in apoptosis-resistant (preovulatory follicle) cells or attenuate the progression of oligonucleosome formation in apoptosis-sensitive (6–8-mm-diameter follicle) granulosa cells.

The results of these studies show that IL-1β treatment is ineffective in altering the level of oligonucleosomes in hen granulosa cells that are either inherently susceptible (6–8-mm follicles) or resistant (F1 follicles) (Fig. 5). This lack of effect of IL-1β on apoptosis is in agreement with results from studies conducted with whole incubated rat follicles (Flaws et al., 1995). It is also a possibility that recombinant murine IL-1β is not physiologically active in the chicken and/or that chickens do not produce IL-1β, but these are considered less likely as, under comparable conditions, another mammalian cytokine, recombinant murine tumor necrosis factor α, was found to be active and promote oligonucleosome formation in 6–8-mm follicle granulosa cells (Witty et al., 1996). Moreover, IL-1β-like activity has been reported by several investigators [for a review, see Klasing and Johnstone (1991)], and a chicken IL-1β receptor with considerable amino acid homology to the mammalian receptor has been cloned and characterized (Guida et al., 1992). Thus, it is concluded from the above experiments that IL-1β, the putative sole product of caspase-1 activity, is not directly involved in modulating hen granulosa cell viability. By comparison, transfection of chicken dorsal root ganglion cells with a dominant negative inhibitor of caspase-1 activity (ICE^{C285G}) was found to inhibit apoptosis induced by trophic hormone withdrawal (Friedlander et al., 1997). Such results suggest an important role for caspase-1 and mature IL-1β in modulating cell viability in this tissue. Although not tested herein, it is possible that expression of caspase-1 in hen ovarian follicle tissue is related to a role for IL-1β in the process of ovulation, as has been previously suggested for mammalian species (Adashi, 1997).

4. Conclusions

(1) The nucleic acid and deduced amino acid sequences of chicken caspase identified in the present study are most closely related to the caspase-1 subfamily of mammalian caspases, and show 60.8 and 44.9% identity, respectively, with the human caspase-1 sequences.

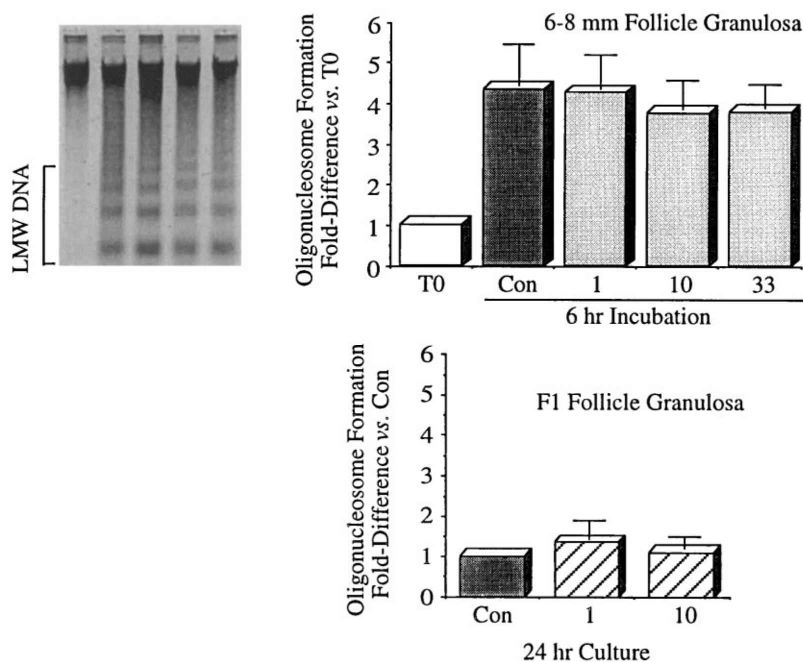

Fig. 5. (Left) Representative SYBR Green-stained gel of oligonucleosome formation in granulosa cells from 6–8-mm follicles. LMW, low-molecular-weight DNA quantitated by densitometry. (Right) Granulosa cells from 6–8-mm follicles undergo spontaneous apoptosis during suspension incubation. However, interleukin-1β (IL-1β) treatment (1, 10, 33 ng IL-1β/ml medium) fails to alter oligonucleosome formation in the 6–8-mm follicle (top) or F1 follicle (bottom) granulosa cells compared to control (Con) incubated or cultured cells. Considering that IL-1β is the only known product of caspase-1 activity (Wilson et al., 1994), it is concluded that caspase-1 is not an integral part of the apoptotic cascade in hen granulosa cells. The level of oligonucleosome formation is expressed as a fold-increase versus the appropriate control (Johnson et al., 1996). T0, unincubated cells.

(2) The chicken caspase-1 protein is predicted to contain a relatively short N-terminal prodomain terminating with Asp^{19}, and encodes Cys^{162} within the active site pentapeptide ($QC^{162}CRG$); this latter finding represents a unique aa substitution compared to chicken caspase-2 and all mammalian caspases identified to date.
(3) Of the hen tissues evaluated, levels of the 1.7-kb caspase-1 transcript were determined to be highest in the bursa of Fabricius and lowest in the cerebrum, pituitary and ovarian follicle granulosa cell layer.
(4) Considering the relatively low levels of caspase-1 mRNA expression in ovarian follicle tissues plus the inability of IL-1β to either promote or attenuate oligonucleosome formation in granulosa cells, in vitro, it is concluded that caspase-1 activity is not an integral component of the apoptotic cascade within granulosa cells that results in ovarian follicle atresia.

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