Overexpression of CRIP in transgenic mice alters cytokine patterns and the immune response

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Received 12 November 2001; accepted in final form 7 February 2002

Cysteine-rich intestinal protein (CRIP) is a zinc-binding protein that was initially identified as a developmentally regulated intestinal gene and has subsequently been identified in several other tissues and cells (3, 15, 16). The zinc-binding property of CRIP has been attributed to the LIM domain, which consists of a specific two zinc finger motif that has a highly conserved Cys2HisCys plus Cys4 sequence (9, 13). The LIM protein family is large. CRIP is the most elementary member of the Group 2 LIM protein family, because it contains only one LIM domain. This group within the LIM protein family does not contain a homeodomain as found in other LIM proteins. In general, it is proposed that the LIM protein family plays a regulatory role that influences differentiation and growth of eukaryotic cells. However, the exact function of any of the Group 2 LIM proteins is not established. LIM proteins may increase in relative abundance with increasing genomic complexity of the organism. For example, in Saccharomyces cerevisiae and Caenorhabditis elegans, the LIM domain genes represent 2 and 6% of all zinc finger domain genes in the respective genomes (9). This increasing abundance points to key regulatory functions for all LIM proteins.

CRIP expression is upregulated in the intestine of young rats just before weaning (3, 24). We proposed that CRIP expression in neonates is induced by glucocorticoid hormones (24). Indeed, the CRIP promoter has consensus sequences for glucocorticoid response elements and imparts glucocorticoid responsiveness in reporter constructs, and glucocorticoid hormone will induce precocious CRIP synthesis in newborn rats (23, 24). Consequently, glucocorticoid hormones most likely contribute in part to the postnatal increase in expression.

Our experiments have demonstrated that CRIP is expressed in immune cells and tissues, specifically in peritoneal macrophage and peripheral blood mononuclear cells, at levels similar to the high levels of expression in the small intestine of weanling and adult rats (15, 24). Moderate expression has been observed in the lung, spleen, heart, and thymus, with very low expression in liver, brain, and kidney (12, 15). The tissue specificity of CRIP expression plus its upregulation during early postnatal development suggest that this LIM protein may have a function in cells involved in host defense.

To further study the function of CRIP, we developed a line of transgenic mice that overexpress the rat CRIP (rCRIP) gene in a tissue-specific fashion (12). Because CRIP is highly expressed in immune tissues, we challenged these mice with both influenza virus (intracellular pathogen) and lipopolysaccharide (LPS; extracellular pathogen) and lipopolysaccharide (LPS; extracellular pathogen).
lular pathogen) to evaluate the effect of CRIP overexpression on two different types of host immu-
nity. In this study, we report that mice that produce more CRIP are more sensitive to an endotoxic chal-
lenge and influenza virus and exhibit an altered cyto-
enkine production pattern. Both findings are consistent with a regulatory function for CRIP, particularly in immune cells.

MATERIALS AND METHODS

Animals and experimental design. A 15-kg rat genomic clone was isolated and subjected to multiple restriction en-
zyme digests. Subsequently, a 5-kb fragment containing the rCRIP gene was ligated into sites of a pGEM cloning vector to transform competent cells. Appropriate colonies were grown and, after rounds of nested deletions and sequencing, PCR primers were designed and used to amplify an ~5-kb fragment. This PCR product contained the rCRIP gene, including 2,644 bp of the 5’ flanking regulatory region and 1.8 kb of the structural portion of the rCRIP gene including all five exons, and was used to produce the transgene construct as described in detail previously (12). Because the rat and mouse CRIP cDNAs, and thus presumably the genes, are highly homolo-
gous, a 91-bp of pGEM vector sequence was included in the construct for the purpose of transgene identification in trans-
genic (Tg) mice. The responsiveness of the rCRIP promoter in a reporter construct in IEC-6 transfected cells has been previously described (23).

Homozgous Tg mice were developed in the B6SJL hybrid strain from the founder line exhibiting the greatest CRIP expression (12). After the mating of F1 animals, which carried the transgene (both female and male), the expected ratio of offspring (1:2:1; noncarriers-heterozygous-homozygous) was obtained. Determination of transgense copy number by the slot blot technique was described previously (12). Het-
eryzygous mice had ~5 copies of the transgene, whereas the homozgous animals had ~8 copies. Homozgous littermates were then bred to maintain the transgenic line.

Some of the mice used in these studies, both Tg and wild-type (NTg), were born in a specific pathogen-free (SPF) mouse facility. They were allowed free access to autoclaved, deionized water and an irradiated commercial diet (Teklad Mouse Breeder Sterilizable Diet 7904; Harlan, Madison, WI). Tg and NTg mice were also bred and maintained in conven-
tional housing conditions and were provided tap water and a commercial rodent diet (Teklad 8604). The mice were age-
matched (4–12 wk of age, except the neonatal study) and, where possible, were sex-matched for experiments.

There were no significant differences in body weights or organ weights between the Tg and NTg mice from either environment (data not shown). In some experiments, Tg and NTg mice from the conventional environment were injected with LPS (Escherichia coli Serotype 0127:B8; Sigma Chemical (St. Louis, MO); 20 mg/kg body wt ip) or saline at various times before being killed. In separate experiments, mice were infected intranasally with virus (Influenza A/Port Chalmers/1/73[H3N2]) (39). Delayed-type hypersensitivity (DTH) re-
tions of mice were evaluated in another series of experiments by application of 2,4-dinitro-1-fluorobenzene (DNFB, 50 μl; 0.5% in 4:1 acetone-oil vehicle) to the shaved abdomen on two successive days (25). Four days after the second application, the mice were rechallenged with a 0.2% DNFB application (20 μl to the left pinna and 20 μl of acetone-oil vehicle to the right). Twenty-four hours later, swelling of pinnae was measured with a micrometer. The DTH response was ex-
pressed as the percent change in pinna thickness after DNFB challenge. The mice were anesthetized with methoxyflurane, halothane, or pentobarbital in various experiments. All ani-
mal procedures were approved by the University of Florida Institutional Animal Care and Use Committee.

Tissue processing. Zinc concentrations of serum derived from blood obtained by cardiac puncture were measured by atomic absorption (15). The proximal duodenum of the small intestine was excised and flushed with ice-cold 0.9% saline. Mucosal cells were removed by scraping and homogenized with a Potter-Elvehjem homogenizer in 4 volumes of 20 mM HEPES, pH 7.4, 1 mM EDTA, and 300 mM mannitol containing 5% protease inhibitor cocktail (Sigma) added just before use. A 40,000-g cytosol fraction from these homog-
enates and those from spleen and thymus were similarly prepared, as described previously (16, 24). Samples of small intestine, spleen, and thymus were also immediately homog-
enized in TriPure Isolation Reagent (Roche Molecular Bio-
chemicals, Indianapolis, IN) with a polytron (Brinkman, Westbury, NY) to isolate total RNA. Protein concentration of the cytosol was determined colorimetrically (27).

mRNA was collected from the mucosa-infected mice and treated as described below. Phytohemagglutinin (PHA)-
stimulated cytokine production used splenocytes isolated by passage of minced spleen fragments through nylon mesh. Cells were washed with RPMI (Mediatech, Cellgro, Herndon, VA)-complete medium (RPMI-1640 supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 2 mM l-glutamine, 25 mM HEPES buffer, and 50 μM 2-mercaptoethanol). After the cells had been washed for a total of three washes in RPMI-complete, they were suspended in RPMI-complete with 10% heat-inactivated fetal calf serum at 2 × 10^6 cells/ml. An aliquot of cells was incubated with 10 μg/ml of PHA in 48-well cell culture plates for 24 h at 37°C, 95% humidity, and 5% CO₂. The supernatants were removed and frozen at –80°C until the cytokines were measured, as we will de-
scribe.

Quantitative PCR and Western analysis. cDNA was syn-
thetized from 1–3 μg of total RNA using reverse transcriptase (RT) and amplified with DNA polymerase in a 25-μl reaction mixture. Measurement of rat or mouse (r/m)CRIP mRNA levels by quantitative PCR (Q-PCR) used primers (forward) 5’-GGGCAAGGAGGATTTTCA-3’ and (reverse) 5’-TTCACCATTTCCTGCACCTC-3’, and a fluorescence res-
sonance energy transfer probe 5’-6FAM-TGACGTCATAG-
GCAAAGCTGCGAT-TAMRA, selected with Primer Express software (Applied Biosystems, Foster City, CA). These were based on sequence information for rCRIP (12). The primer and probe set for 18S ribosomal RNA used for nor-
malization were from Applied Biosystems. Relative quanti-
tication was calculated from a standard curve generated by 1:10 dilutions of total RNA to produce a 4- to 5-log range as described recently in detail (28). Amplification of PCR prod-
ucts was measured fluorometrically (ABI model 5700 Se-
dence Detection System). Routinely, RT reactions were for 30 min at 48°C, and PCR was for 40 cycles.

Proteins in tissue cytosol preparation were separated by 15% Tris-Tricine SDS-PAGE (16). After transfer to Immob-
ilon-P membrane (Millipore, Bedford, MA), immunodetection was with ammonium sulfate precipitate and protein A-purified rabbit IgG prepared against a synthetic peptide (CGFGRGGAESHTFK) representing amino acids 65–77 of the COOH terminus of r/mCRIP with a cysteine added to facilitate conjugation to keyhole limpet hemocyanin. These methods have been described previously (14, 20). A second-
ary antibody-alkaline phosphatase conjugate and an extra-
cellular fluid reagent (Amersham Pharmacia Biotech, Piscataway, NJ) were used for fluorescence imaging (Molecular
Dynamic Storm 840 Imager). Recombinant human CRIP, prepared with an expression system described previously (20), was used as the standard.

**Lung viral titers/cytokine assays.** Viral titers were determined by inoculation of ground lung samples into Madin Darby canine kidney cells, as described previously (39). Splenocyte IFN-γ, IL-6, IL-10, and tumor necrosis factor-α (TNF-α) after PHA challenge, as well as serum IFN-γ, IL-6, IL-10, and TNF-α after LPS challenge, were measured using commercial ELISA kits (Cytimmune, College Park, MD). IL-2 production was based on [3H]thymidine incorporation by murine HT-2 lymphocytes measured using a bioassay, as described previously (22).

**Statistical analysis.** Tests for significance were performed using the Student’s t-test, the Mann-Whitney test, or 2 × 2 factorial ANOVA with GraphPad Software (San Diego, CA) or the SAS System (SAS Institute, Cary, NC). When necessary, data were subjected to log10 transformation to lessen variance heterogeneity.

**RESULTS**

**Response to the environment.** Relative quantitative analysis of CRIP mRNA quantities (abundance) by Q-PCR shows the wide range of CRIP expression that occurs in neonates from birth to 41 days of age (Fig. 1). Of note is that SPF-maintained NTg mice exhibited essentially the same CRIP upregulation, starting at ~15 days of age, as did conventionally maintained mice. Also of considerable interest is that CRIP mRNA levels in thymus and spleen are negligible during these early weeks of life (Fig. 1).

**Response to LPS challenge.** Because CRIP expression is highly expressed in immune cells, which respond to microbial factors, we subjected both the NTg and Tg mice to LPS as a model extracellular pathogen challenge. The induction of CRIP expression by LPS is shown in Fig. 2. CRIP mRNA was measured by real-time PCR, and data are presented as relative quantities (Fig. 2A). LPS produced increases in CRIP mRNA ranging from 1.4 to 2.2 in intestine, spleen, and thymus. These were statistically significant (*P* ≤ 0.02) for each comparison pair (±LPS). Expression was markedly greater in the Tg mice. Similarly, Western analysis showed that LPS induced changes in CRIP protein that were present in cytosol extracts of these tissues. However, the sensitivity was such that only the data from the Tg mice were analyzed statistically (Fig. 2B). As shown, LPS produced a significant increase for each comparison pair (*P* ≤ 0.05).

Reduced serum zinc concentrations are a well recognized consequence of acute endotoxemia and demonstrate here the comparable systemic response of NTg and Tg mice to LPS (Fig. 3A). Within 48 h after LPS challenge, over 50% of Tg mice died, in contrast to only...
Serum cytokines after LPS challenge and PHA-stimulated cytokine release by splenocytes from nontransgenic and CRIP transgenic mice

During the course of the challenge, all Tg mice developed diarrhea (Fig. 3C). These mice were lethargic and did not groom themselves, both features of endotoxic shock (26). In comparison, morbidity was less in the NTg mice. Tg mice also lost significantly more weight than NTg mice by day 2 after LPS (Fig. 3D). After this point, NTg mice began to recover from the LPS, as observed by increased weight. At the end of the 8-day comparison period, the Tg mice still had a lower body weight.

Saline-treated NTg and Tg mice did not lose weight, and there was no mortality (Fig. 3, B and D). Because intestinal CRIP expression was greater in Tg mice than in NTg mice, the higher CRIP level is correlated to the higher incidence of LPS-induced diarrhea in the Tg mice after LPS challenge.

The increased sensitivity to LPS observed in the Tg mice suggests that the response could be the result of cytokine dysregulation. To investigate this possibility, serum cytokines were measured after LPS challenge (Table 1). Serum IFN-γ concentrations in Tg mice were about one-third of those found in the NTg group. Serum TNF-α was also depressed in the two genotypes, but not significantly. Serum IL-10 was approximately threefold higher in Tg mice, and serum IL-6 was twofold higher in these mice. These results are consistent with an increase in cytokine dysregulation in the CRIP-overexpression mice after a stimulus of an extracellular pathogen. To further investigate the possibility of cytokine dysregulation in CRIP overexpression, splenocytes obtained from both genotypes were stimulated in culture with the mitogen PHA (Table 1). There was significantly less IFN-γ and IL-2 production by the cultured Tg splenocytes, with correspondingly greater IL-6 and IL-10 production. There was no difference in TNF-α production between splenocytes from the two genotypes. Splenocytes from Tg mice also exhibited significantly (P < 0.008) decreased proliferation (24% of the NTg cells) using the [3H]thymidine incorporation assay (data not shown).

The observed increases in Th2 cytokines IL-6 and IL-10 observed in the CRIP Tg mice suggest that they would show an altered Th1 response, e.g., DTH. As shown in Fig. 4, the DTH response to DNFB was significantly depressed in the Tg mice. This supports the finding of cytokine imbalance favoring Th2 over Th1 cytokines in the Tg mice.

Response to influenza virus. Infection with influenza virus served as a model to test the effect of CRIP overexpression on the response to an intracellular pathogen. Serum zinc concentrations at day 2 were comparable in the two genotypes, but there was a significant decline in the Tg mice by day 8 (Fig. 5A).

Table 1. Serum cytokines after LPS challenge and PHA-stimulated cytokine release by splenocytes from nontransgenic and CRIP transgenic mice

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-2, U/dl</th>
<th>IFN-γ, ng/ml</th>
<th>IL-10, pg/ml</th>
<th>IL-6, pg/ml</th>
<th>TNF-α, pg/ml</th>
</tr>
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<tbody>
<tr>
<td>Serum cytokines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontransgenic</td>
<td>65 ± 11</td>
<td>756 ± 130</td>
<td>908 ± 422</td>
<td>173 ± 40</td>
<td></td>
</tr>
<tr>
<td>Transgenic</td>
<td>24 ± 5</td>
<td>2,082 ± 332</td>
<td>1,211 ± 511</td>
<td>90 ± 24</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.005</td>
<td>0.003</td>
<td>0.03</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Splenocyte cytokines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontransgenic</td>
<td>69 ± 2</td>
<td>102 ± 31</td>
<td>7 ± 2</td>
<td>365 ± 58</td>
<td>411 ± 47</td>
</tr>
<tr>
<td>Transgenic</td>
<td>56 ± 2</td>
<td>19 ± 7</td>
<td>17 ± 4</td>
<td>660 ± 56</td>
<td>401 ± 49</td>
</tr>
<tr>
<td>P value</td>
<td>0.03</td>
<td>0.005</td>
<td>0.003</td>
<td>0.03</td>
<td>NS</td>
</tr>
</tbody>
</table>

Nontransgenic (control) and cysteine-rich intestinal protein (CRIP) transgenic mice were maintained under conventional housing conditions. Serum cytokines from both genotypes were measured 24 h after the mice received E. coli lipopolysaccharide (2 mg/kg/ip). Cytokine release into culture medium was measured by 24 h after addition of phytohemagglutinin (PHA, 10 μg/ml) to cultures of splenocytes derived from mice of both genotypes. Interleukin (IL)-2 was measured by [3H]thymidine incorporation. Other cytokines were measured by ELISA. IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; NS, not significant. Values are means ± SE of 6–8 mice/group. P values represent differences between means from nontransgenic and transgenic mice.
The ability to clear influenza virus from the lung was significantly compromised \((P < 0.05)\) in the Tg genotype (Fig. 5B). By day 8, all of the NTg mice, but only one of the Tg mice, had cleared the virus from the lungs \((P < 0.05)\). All mice began to lose weight during the first 24 h after infection (Fig. 5C). After day 3, the NTg mice started to regain weight. The Tg mice continued to lose weight through day 4 and had significantly \((P < 0.05)\) lower body weight throughout the 8-day observation period. The reduced response to viral challenge in the Tg mice points to an altered host defense due to CRIP overexpression.

DISCUSSION

The normal physiological role of CRIP is not known. However, the high level of expression in immune cells compared with other cells is evidence that this protein may have a role in host defense \((14, 15, 20)\). In support of this notion, high levels of CRIP expression in peritoneal macrophages, thymus, and spleen are increased upon induction by LPS \((14, 15)\). Intestinal CRIP abundance varies among intestinal cell types, being highest in Paneth cells, followed by eosinophils within the lamina propria \((14)\). Both cell types are well defined immune cells. CRIP is also localized, albeit to a lesser extent, in intestinal epithelial cells \((14)\). Recently, the gut epithelium has been shown to produce inflammatory cytokines in response to pathogenic bacteria \((33)\). Consequently, enterocytes must also be considered to be immune cells, perhaps by aiding in tolerance of the gut to pathogens. To further test the possibility of an immune function for CRIP, in these studies we examined the result of CRIP overexpression to models of extracellular (LPS) and intracellular (viral infection) pathogen exposure.

The intestine provides an important part of the mucosal immune system \((6, 8, 18)\); consequently, factors regulating this system should respond to conditions within the intestinal lumen. This time period corresponds to the point at which the gut is adapting to both colonization with enteric microflora and marked changes in diet \((17, 18)\). As shown in Fig. 1, the developmental increase in CRIP expression is not a response to environmental factors, because CRIP concentrations increase to the same extent in neonates maintained in SPF condition as in those raised in a conventional environment. This suggests that glucocorticoid hormone, which is able to produce a precocious increase in CRIP expression in neonatal rat pups \((24, 32)\), is the primary stimulus for the developmental increase in CRIP in rodent intestine. An immunological response to enteric pathogens may be a factor in CRIP expression, but that possibility was not tested in these experiments.

Our Tg mice maintained in an adult breeding colony have not shown any abnormality in reproduction, growth, or gross intestinal morphology; however, as shown in this report, these mice are more sensitive to LPS and influenza virus. This suggests to us that the function of CRIP is as a modifier of a response rather than as a direct initiator of a response, which, if overexpressed, would result in obvious morbidity or morphology.

Fig. 4. Delayed-type hypersensitivity response of CRIP Tg and NTg controls. Male and female mice were challenged with DNFB, as described in MATERIALS AND METHODS. The right pinna was treated with the vehicle; the left pinna was treated with DNFB. Data are expressed as the %change in thickness of the DNFB vs. vehicle-treated pinnae from NTg and Tg mice. Values are means ± SE \((n = 26)\). Asterisk indicates that the value is significantly different \((P < 0.05)\) from that of NTg mice.

Fig. 5. Response of CRIP Tg and NTg control mice to influenza virus. Mice (males) were challenged intranasally with influenza virus, as described in MATERIALS AND METHODS. Values shown are means ± SE; \(n = 3\) group. A: serum zinc concentration; B: lung viral titers on days 2, 4, and 8 postinfection; C: %change from initial body weight after influenza virus infection. Asterisks indicate that values are significantly different \((P < 0.05)\) from NTg mice.
LPS is known to cause a complex reaction of the immune system (36) and initially has strong stimulatory effects on macrophages and other immune cells, including release of cytokines, such as IL-1, IL-6, and TNF-α. The time period chosen to examine these cytokines was one when some, e.g., IL-2, exhibit maximal levels after stimulation. CRIP overexpression appears to alter cytokine patterns in LPS-stimulated mice with a shift to an increase in IL-6 and IL-10 and a decrease in IFN-γ and IL-2. Production of TNF-α, a macrophage-derived cytokine, is not changed with CRIP overexpression within the time period examined, suggesting that differences in production of these cytokines is somewhat specific and that CRIP overexpression affects lymphocyte function more than monocyte function. Because IL-10 suppresses lymphocyte and macrophage function, the marked (threefold) increase in its production may explain, in part, the increased sensitivity to LPS that the Tg mice display.

Evaluation of splenocyte proliferation and cytokine production of mitogen-challenged splenocytes provided additional support for the hypothesis that CRIP overexpression influences cytokine balance. This hypothesis is further supported by the decreased ability of the Tg mice to clear virus. As with aging mice, there appears to be a decrease in IFN-γ and IL-2 and an increase in IL-6 and IL-10 in response to this immune challenge in Tg mice (39). The decrease in Th1-associated cytokines, including IFN-γ and IL-2, in aged mice is related to a decreased ability to clear influenza virus from the lung (39). Additionally, Th2 cytokines such as IL-6 and IL-10 may cause delayed virus clearance in influenza-infected mice (29).

To balance the immune response, there is cross-regulation between the cytokines that affect immune cells, such as Th1/Th2 cells (10, 30). For example, IL-4 drives Th2 T cell differentiation, and IFN-γ and IL-12 drive Th1 T cell differentiation. In addition, IL-4 and IFN-γ exert antagonistic effects on Th1 and Th2 processes, respectively (35). Thus, from the data collected in this group of experiments, two questions arise: 1) is CRIP overexpression affecting macrophage or T-helper cell function, i.e., upregulating Th2 cell functions and downregulating Th1 cell functions; or 2) is CRIP overexpression affecting the differentiation pathways of these two cell types? CRIP expression may also be related to a combination of these two events. Using the data presented here, we have developed a working model, briefly described earlier (11), based on CRIP-related cytokine dysregulation that will be tested in future experiments. There is precedence in the literature for cytokine expression regulation by a zinc finger protein. Specifically, TNF-α production is regulated by tristetraprolin, a CCCH zinc finger protein (7), through a feedback inhibitory mechanism influencing TNF-α mRNA.

Our model of CRIP function currently focuses on the decreased levels of IFN-γ and IL-2 secreted by CRIP-Tg mice described in this report. This emphasis is supported by other evidence, including: 1) presence of consensus response elements in the CRIP promoter (23) necessary for IFN-γ-induced gene expression; 2) protein-protein interaction observed in vitro (J. Nicewonger and R. Cousins, unpublished data) between CRIP and the IFN-γ chaperone calreticulin (21); and 3) zinc inducibility of calreticulin through metal response elements in the calreticulin promoter (34). This model will be applied to test the response of CRIP function in other situations, e.g., zinc deficiency. Th2 cytokine production is decreased in zinc-deficient mice (37). The same response has been shown in zinc-deficient humans (2). Because CRIP is a zinc finger protein that may be influenced by cellular zinc pools, a link between zinc-related immune dysfunction and the proteins CRIP and calreticulin will be a focus of our further research.

Like other LIM proteins, CRIP may be involved in control of cellular determination and differentiation or the regulation of specific processes. The LIM protein MLP is involved in muscle cell differentiation (1), and LMO1 (previously Rbtn1 or Ttg-1) is a transcription factor involved in T cell proliferation (5). Similarly, the protein LMO2 (previously Rbtn2 or Ttg-2) was found to be part of a DNA-binding complex important for mouse hematopoietic development (31, 40, 41). The function of CRIP may involve protein-protein interactions that include interaction with cytoplasmic factors and/or nuclear factors such as the nuclear LIM interactor protein (19) or the Clim/Ldb/Nli coregulator family (38).

In summary, we have shown in these experiments that CRIP overproduction decreases IFN-γ and IL-2 levels and increases IL-6 and IL-10 levels. The imbalance favoring Th2 cytokines suggests that CRIP regulates expression/secretion of these cytokines from cells where this zinc finger protein is very highly expressed. Mice producing larger than normal amounts of CRIP are, therefore, more susceptible to toxins (as shown here with LPS) produced by pathogens and to viral infection. The response of CRIP expression to LPS and viral challenges further strengthens a role for this LIM protein as an adapter molecule for modifying the action of factors associated with cellular mechanisms leading to dysregulation of cytokines required for cellular host defense.

We express our appreciation to Warren R. Clark, Robert J. Cottey, Leah M. Coy, and Kelli A. Herrlinger-Garcia for help with some analyses.

This research was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-31127 (R. J. Cousins), Boston Family Endowment Funds (R. J. Cousins), and Department of Veterans Affairs (B. S. Bender).

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