The 5'-Flanking Region of the Human CGL-1/Granzyme B Gene Targets Expression of a Reporter Gene to Activated T-lymphocytes in Transgenic Mice*

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The human CSP-B/CGL-1 gene is the homologue of the mouse granzyme B/CCPI gene and encodes a cytotoxic T-lymphocyte-specific serine protease. We have used regulatory sequences upstream from the CSP-B gene to drive human growth hormone gene expression in transgenic mice. Eleven founder mice were screened for transgene expression in activated T-cells. Expression was detected in 10 mice; levels of expression were integration site-dependent. The transgene was not expressed in resting lymphocytes but could be activated by treatment with concanavalin A or interleukin-2, indicating that CSP-B regulatory sequences are responsive to signals originating at either the T-cell receptor or the interleukin-2 receptor. Transgene expression was detected at the whole organ level only in lymph nodes and small intestine, where endogenous mouse CCPI mRNA was also present. The time course of transgene activation in T-lymphocytes was similar to that of the mouse CCPI gene. No differences in levels of expression of the transgene were observed in activated lymphocyte populations that had been depleted of either CD4+ or CD8+ cells; in contrast, the mouse CCPI gene was expressed primarily in CD8+ cells. Six CD4+ T-cell clones with Th0, Th1, or Th2 phenotypes were generated from a transgenic animal. All clones expressed moderate to high levels of the transgene, but only three clones expressed mouse CCPI, indicating that the transgene is disregulated in CD4+ T-cell subsets. The CSP-B regulatory unit represents a novel reagent for targeting gene expression to activated T-lymphocytes.

The activation of T-lymphocytes in vivo occurs after binding of antigen and self-histocompatibility molecules to the T-cell receptor (1–3). A cascade of gene regulatory events ensues, leading to the acquisition of a phenotype, such as cytotoxicity. The activation process occurs over a period of several days, but the complex series of events leading to the acquisition of immunologic functions are not yet understood. A great deal of work has recently focused on early aspects of T-cell activation. Several second messenger systems triggered by T-cell receptor occupancy have been identified (4–6) and are presumably linked to the activation of so-called “immediate-early” genes (such as c-myc and c-fos) (7, 8). However, a number of gene activation events occur up to several hours or even days after the initial triggering stimulus (reviewed in Ref. 9). Understanding the regulation of these “late” genes may allow us to reconstruct the regulatory network further.

We recently described an hematopoietic serum protease gene cluster on chromosome 14q11.2 (10). This locus contains the myeloid-specific gene cathepsin G (11, 12), the lymphokine-activated killer cell- and natural killer cell-specific gene CGL-2 (also known as CCP-X or granzyme H) (13–15), and the cytotoxic T-cell-specific gene CSP-B/CGL-1 (also known as CCPI, granzyme B, CTLA-1, and SECT-1) (16–21). The CSP-B/CGL-1 gene encodes a serine protease that is found in the cytolytic granules of cytotoxic T-cells (22). These granules contain several proteases, as well as the pore-forming protein perforin (19, 23); these proteins are exocytosed when the cytotoxic effector cell comes in contact with a target cell (24). The role of the granule constituents in the killing process is not yet clear (25, 26). The cytolytic granules of cytotoxic T-lymphocytes are assembled only after antigenic stimulation and require de novo synthesis of the individual granule enzymes (27). In the case of CSP-B/CGL-1, this synthesis occurs because of transcriptional activation of the CSP-B gene (28). We are interested in the regulation of CSP-B/CGL-1 expression as a model for the induction of “late” genes during T-cell activation.

We recently reported that the PEER T-cell leukemia cell line could be induced to express the CSP-B gene by treatment with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) in combination with N2'-O-dibutyryl cyclic AMP (28). We demonstrated that the CSP-B promoter and 5'-flanking sequences were necessary and sufficient to reconstitute this activation response in a transient transfection assay. To further examine the tissue specificity and inducibility of the CSP-B regulatory unit, we linked 1.2 kb of 5' CSP-B/CGL-1 flanking sequence to the human growth hormone gene and used this chimeric gene to generate transgenic mice. In this report, we demonstrate that expression of the transgene is targeted specifically to activated T-lymphocytes and is inducible with agents acting either through the T-cell receptor or the IL-2 receptor. The transgene is apparently disregulated in T-lymphocyte subsets, since 1) CD4+ and CD8+ T-cells express approximately equal amounts of transgene mRNA and 2) CD4+ T-cell clones of both Th1 and Th2 subtypes...
consistently expressed moderate to high levels of transgene mRNA. The 5'-flanking region of the CSP-B/CGF-1 gene may therefore be a useful reagent for targeting novel gene products to the activated T-cells of transgenic mice.

**MATERIALS AND METHODS**

**Construction of the CSP-B-hGH Transgene**—The ~1170 CSP-B-neo plasmid (28) was linearized with SaI and partially digested with HindIII. The 3.9-kb fragment containing vector DNA and CSP-B sequences from -1170 to +31 relative to the transcriptional start site was gel-purified. The 2.2-kb genomic human growth hormone BomHI/EcoRI fragment was HindIII-linked at the BomHI site and subcloned in pBluescript. The growth hormone gene was purified following digestion with HindIII and SaI and subcloned into the CSP-B vector. A 3.4-kb fragment free of vector DNA was purified following digestion with EcoRI and was used for microinjection.

**Production of Transgenic Mice**—Transgenic mice were generated as described (29). Briefly, the CSP-B-hGH fragment was resuspended in microinjection buffer (5 mM Tris, pH 7.4, 0.25 mM EDTA, and 5 mM NaCl) at 2 ng/μl. C57BL/6 F1 females were superovulated by sequential intraperitoneal injections of 5 units of pregnant mare serum (Sigma) and 5 IU of human chorionic gonadotropin (Sigma) and mated with fertile F1 males. Single-cell embryos were harvested the following day using Brinster’s BMMC-3 medium (Gibco Laboratories). Cumulus cells were removed by brief incubation in 300 μg/ml hyaluronidase. Pronuclei were microinjected with approximately 1–2 pl of CSP-B-hGH DNA, and two-cell embryos were reimplanted the following day into pseudopregnant Swiss Webster outbred female mice.

**Lymphocyte Activations**—For activation of tail blood lymphocytes, 50 μl of heparinized blood was mixed with 12 ml of Iscove’s modified eagle medium (GIBCO) containing 10% fetal calf serum (GIBCO), 10^-4 M β-mercaptoethanol, 50 μg/ml gentamicin sulfate (U. S. Biochemical Corp.), 100 units/ml penicillin G, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, 2 μg/ml ConA, 25 units/ml human recombinant IL-2 (rIL-2), and 25 × 10^-5 irradiated (2000 centigrays) C57BL/6 splenocytes. The mixture was divided among wells of a 96-well plate and cultured for 4 days at 37 °C in 5% CO2.

Lymphocyte activations were performed using either whole spleen suspensions or Ficoll-Hypaque-purified splenic lymphocytes. Cells were cultured in Iscove’s modified eagle medium containing fetal calf serum, β-mercaptoethanol, and gentamicin as above. ConA and/or rIL-2 were added at the concentrations indicated in the figure legends.

**RNA Purification and Analysis**—RNA was purified with a guanidinium thiocyanate extraction procedure as described previously (30). Preparation of end-labeled probes and S1 nuclease protection assays were performed as previously described (31). The murine β2-microglobulin probe is a genomic fragment labeled at the EcoRI site in exon 2. The murine CCPI probe is a genomic fragment labeled at the BomHI site in exon 5.

**Generation of T-cell Clones and Assay for Lymphokine Production**—Mice were immunized subcutaneously with 100 μg of hen egg lysozyme (HEL) in complete Freund’s adjuvant. One week after immunization, draining lymph nodes were harvested and single cell suspensions were prepared. Cells were stimulated with HEL (100 μg/ml) in 20 ml of 5% fetal calf serum/Dulbecco’s modified Eagle’s medium in T25 culture flasks (upright position). After 7 days of in vitro culture, cells were recovered and cloned at 1 cell/well in the presence of 5 × 10^5 irradiated (2000 centigrays) CBA/J spleen cells, 100 μg/ml HEL, and 50 units/ml murine rIL-2 in a final volume of 200 μl of 10% fetal calf serum/Dulbecco’s modified Eagle’s medium in round bottom microtiter wells. Growing cells were expanded and maintained in 20-ml cultures containing CBA/J spleen cells, HEL, and rIL-2. Cells (5 × 10^5/ml) were stimulated with 5 μg/ml ConA for 24 h, and supernatants were harvested. IL-2 and IL-4 activities were measured in the presence of either anti-IL-2 antibody (4B6) or anti-IL-4 antibody (11B11) using a cell line capable of responding to both IL-2 and IL-4, as described previously (32).

**RESULTS**

**Generation of CSP-B-hGH Transgenic Mice**—We have previously shown that CSP-B sequences between -1170 and +51 (relative to the transcription start site) are necessary and sufficient for inducible expression of a linked reporter gene in the PEER cell line (28). We therefore linked this regulatory unit to the human growth hormone gene, creating the CSP-B-hGH construction shown in Fig. 1. A probe end-labeled at the Smal site in the 3'-untranslated region of the growth hormone gene was used to detect CSP-B-hGH expression using an S1 nuclease protection assay. A 3.3-kb EcoRI fragment containing the chimeric gene was injected into fertilized C57BL/6 × C3H eggs. Eleven founder transgenic mice were produced from a total of 53 live births.

We initially screened tail blood from all of the transgenic founders to identify those that expressed the transgene in activated T-lymphocytes. Fig. 2A shows the result of this analysis. Whole blood was mixed with irradiated feeder cells and cultured in the presence of ConA and IL-2. RNA was isolated and hybridized with end-labeled probes for hGH and murine CCPI (mCCPI, the homologue of human CSP-B). The mCCPI probe is a genomic DNA fragment end-labeled at the BomHI site in the 3'-untranslated region. Correctly spliced mRNA probe is a genomic fragment labeled at the EcoRI site in exon 2 of human growth hormone. The CSP-B-hGH transgenic mice were generated from 11 founders, and the transgene copy number of each founder was determined by Southern blot analysis. The amount of transgene copy number is shown in Fig. 2B.
mCCPI mRNA protects a 164-nt fragment derived from this probe. Correctly spliced CSP-B-hGH mRNA protects a 204-nt hGH probe fragment. Ten founder mice have detectable levels of transgene expression (Fig. 2, lanes 1–10). The ratio of CSP-B-hGH to mCCPI mRNA was calculated for each founder mouse by densitometric scanning. The transgene copy number in the individual founder mice was determined by Southern blot analysis (Fig. 2B). Table I summarizes these results, as well as the copy number–corrected level of CSP-B-hGH expression in each founder mouse. There is no correlation between transgene copy number and level of expression, suggesting an influence of integration sites on levels of transgene expression.

Expression of the CSP-B-hGH Transgene Is Inducible and Tissue-specific—Previous studies of CSP-B and mCCPI expression have demonstrated that no expression is detected in resting lymphocyte populations derived from peripheral blood or spleen (28, 34). We therefore tested for inducibility of the transgene in splenic lymphocytes. Founder mice numbers 7, 9, 11, and 30 were bred, and transgenic F1 offspring were used for further studies. Fig. 3 shows an analysis of CSP-B-hGH and mCCPI mRNA levels in spleen-derived lymphocytes from three founder lines before and after 2 days of culture in the presence of ConA and IL-2. No CSP-B-hGH mRNA is detected in the untreated lymphocyte populations (lanes 1, 3, and 5). In contrast, levels of CSP-B-hGH mRNA are comparable with those of mCCPI in activated lymphocytes from founder lines 7 and 9 (lanes 2 and 4), but are barely detectable in founder line 11 (lane 6), in agreement with the results shown in Fig. 2. A β2-microglobulin probe was used to demonstrate RNA quality and content for each sample.

We next analyzed the tissue distribution of CSP-B-hGH and mCCPI mRNA in founder line 30. Fig. 4 shows an S1 nuclease protection assay performed on RNA isolated from various organs of an F1 mouse. Probes specific for CSP-B-hGH, mCCPI, and murine β2-microglobulin were used to detect expression of these genes in the indicated tissues. In lane 1, RNA from ConA + IL-2-stimulated lymphocytes is used as a positive control for CSP-B-hGH and mCCPI expression. No CSP-B-hGH or mCCPI mRNA is detected in heart, liver, stomach, spleen, kidney, brain, or bone marrow (lanes 3, 4, 5, 6, and 7–11). Very low levels of CSP-B-hGH and mCCPI mRNA are present in the proximal small intestine (lane 5), and higher levels are detected in the distal small intestine (lane 6). This may reflect the increase in lymphoid “mass” in distal versus proximal small intestine; only discrete lymphocytes are found in the mucosa of the jejunum (proximal), whereas large clusters of lymphocytes are present in the Peyer’s patches of the ileum (distal). Expression of mCCPI in the small intestine may also correlate with the constitutive cytotoxic activity of intestinal intraepithelial lymphocytes (35). Neither CSP-B-hGH nor mCCPI mRNA is detected in the colon (data not shown). Very low levels of both CSP-B-hGH and mCCPI mRNAs were sometimes detected in mesenteric lymph nodes, although this result was not reproducible within a given founder line. CSP-B-hGH and mCCPI were always coexpressed when expression was detected. A similar analysis of founder lines 7 and 9 gave identical results (data not shown).

CSP-B-hGH and mCCPI Are Activated with Similar Kinetics and Are Inducible Via Either the T-cell Receptor or the IL-2 Receptor—In vivo, T-lymphocyte activation is initiated at the T-cell receptor (1–3). T-cell receptor activation is rapidly followed by synthesis of the IL-2 receptor α-chain and secretion of IL-2 by CD4+ T cells (36). The mCCPI gene can be

![FIG. 3. CSP-B-hGH mRNA is inducible in T-lymphocytes.](image-url)

Lymphocytes were isolated from the spleens of F1 mice produced from the founder lines indicated at the top of the figure. RNA was prepared before (−) or after (+) 2 days of culture in the presence of ConA and IL-2. 10 μg of RNA was hybridized with probes for CSP-B-hGH and β2-microglobulin and analyzed by S1 nuclease protection. Note that CSP-B-hGH mRNA is present in activated, but not resting, lymphocytes.

![FIG. 4. Tissue specificity of CSP-B-hGH and mCCPI expression.](image-url)

RNA was purified from the indicated organs of an F1 mouse produced from founder line 30. Spleen-derived lymphocytes were activated with ConA and IL-2 as a positive control (lymphocytes (+)). 10 μg of RNA was hybridized with probes for CSP-B-hGH, mCCPI, and β2-microglobulin and analyzed by S1 nuclease protection. Note that CSP-B-hGH and mCCPI mRNAs are detected only in activated lymphocytes and in the ileum.
transcriptionally activated by agents acting on the T-cell receptor (34) or by culture in the presence of high concentrations of IL-2 (37). We therefore sought to compare the regulation of CSP-B-hGH and mCCPI in response to different activating stimuli. Fig. 5 shows the result of this experiment, using lymphocytes purified from the spleen of an F1 mouse from founder line 9. Cells were cultured for 1, 2, or 3 days with ConA (lanes 1–3) or for 5 days with 800 units/ml (high dose) recombinant IL-2 (lane 4). RNA was purified and hybridized with probes for CSP-B-hGH, mCCPI, and β2-microglobulin. The time courses of CSP-B-hGH and mCCPI mRNA accumulation in response to ConA are similar. Although mCCPI mRNA is more abundant than CSP-B-hGH mRNA on day 1, expression of both genes can be detected after 1 day and reaches a plateau after 2 days. Activation of the IL-2 receptor with a high concentration of IL-2 also increases CSP-B-hGH and mCCPI mRNA levels (lane 4). Treatment with the B-lymphocyte-specific mitogen lipopolysaccharide did not result in detectable CSP-B-hGH or mCCPI expression (data not shown). We have not attempted to analyze purified B-lymphocytes and therefore cannot exclude the possibility of CSP-B-hGH expression in a subset of these cells. However, CSP-B/CGL-1 regulatory sequences appear to respond appropriately to different stimulatory pathways in the T-lymphocytes of transgenic mice.

Expression of CSP-hGH and mCCPI in T-cell Subsets—Although expression of mCCPI has been observed in all CD8+ T-cell lines analyzed (20, 38, 39), some CD4+ lines of the Th2 subset have also been reported to express mCCPI (40). We therefore compared the inducible expression of CSP-B-hGH and mCCPI in lymphocyte populations depleted of either CD4+ or CD8+ T-cells. Splenocytes derived from a founder line 9 mouse were treated with the monoclonal IgM antibodies L3T4 (anti-CD4) or Lyt2 (anti-CD8) in the presence of rabbit complement. The resulting CD4- and CD8-depleted populations were cultured for 0, 1, or 2 days with ConA and IL-2 and then harvested for RNA. RNA samples were hybridized with probes for CSP-B-hGH, mCCPI, and β2-microglobulin and analyzed by S1 nuclease protection, as shown in Fig. 6A. RNA from the activated CD4- and CD8-depleted populations was also analyzed by Northern blot hybridization with cDNA probes for CD4 and CD8 in order to assess the efficiency of the depletions (panel B). CSP-B-hGH mRNA levels are essentially identical in the CD4- and CD8-depleted populations (compare lanes 2 and 3 with lanes 5 and 6). In contrast, mCCPI mRNA levels are much higher in the CD4-depleted (CD8- enriched) population than in the CD8-depleted (CD4- enriched) cells. B, 10-μg samples of the RNA preparations analyzed in lanes 3 and 6 of panel A were electrophoresed on a formaldehyde gel and analyzed by Northern blot hybridization as described (41), simultaneously using random primer-labeled (42) CD4 and CD8 cDNA probes. Note that the CD4-depleted cells predominantly contain CD8 mRNA and that CD8-depleted cells predominantly contain CD4 mRNA, as expected.

The low level of mCCPI expression in the CD8-depleted (i.e. CD4+) population may be due to contaminating CD8+ cells, or it may be the result of expression in a subset of CD4+ T-cells. To address this question and to determine whether CSP-B-hGH expression was restricted in CD4+ subsets, we generated a panel of CD4+ T-cell clones from a transgenic founder line 9 F1 mouse. Following immunization with hen egg lysozyme, T-cells specific for HEL were cloned and expanded. RNAs from individual clones were analyzed by S1 nuclease protection, as shown in Fig. 7. ConA + IL-2-stimulated splenocytes were used as a positive control for CSP-B-hGH and mCCPI expression (lanes 1 and 5). CSP-B-hGH, mCCPI, and β2-microglobulin RNA levels in the individual clones are shown in lanes 2–4 and 6–8. Supernatants from the individual clones were assayed for IL-2 and IL-4 activity, and the results are shown in the lower part of Fig. 7. The lympho-
was hybridized with probes to clones. Detection only in clones 8, 1, and 12 (lunes detected in all six clones. In contrast, mCCPI mRNA is not detected in any clone were tested for IL-2, IL-4, and T-lymphocytes were subsequently restimulated in vitro with ConA and ionomycin. Supernatants from each clone were tested for IL-2 and IL-4 activity, as described under "Materials and Methods." Note that CSP-B-hGH mRNA is detected in all clones, whereas mCCPI mRNA is only detected in clones 1, 8, and 12. Also note that there is no definite correlation between mCCPI expression and lymphokine secretion patterns. N.D., not done.

Fig. 7. CSP-B-hGH and mCCPI expression in CD4+ T-cell clones. An F1 mouse from founder line 9 was immunized with hen egg lysozyme, and T-lymphocytes were subsequently restimulated in vitro and cloned. RNA was prepared from individual clones 3 days after stimulation with HEL. Bulk splenocytes were activated with ConA and IL-2 as a positive control (splenocytes (+)). 10 μg of mRNA was hybridized with probes for CSP-B-hGH, mCCPI, and β2-microglobulin and analyzed by S1 nuclease protection. Supernatants from each clone were tested for IL-2 and IL-4 activity, as described under "Materials and Methods." Note that CSP-B-hGH mRNA is detected in all clones, whereas mCCPI mRNA is only detected in clones 1, 8, and 12. Also note that there is no definite correlation between mCCPI expression and lymphokine secretion patterns. N.D., not done.

kin secretion patterns of clones 17 and 12 (lanes 4 and 8) correspond to the Th1 T-cell subset (secretion of IL-2 but not IL-4). Clones 8 and 1 secrete IL-4 but not IL-2, corresponding to the Th2 subset. Clone 11 secretes both lymphokines and therefore belongs to the Th0 subset. CSP-B-hGH mRNA is detected in all six clones. In contrast, mCCPI mRNA is detected only in clones 8, 1, and 12 (lanes 2, 6, and 8). There is no definite correlation between mCCPI expression and Th subset. The level of mCCPI mRNA is lower in clone 1 than in the other two mCCPI-expressing clones (compare lane 6 with lanes 2 and 8); clone 1 also has the lowest level of CSP-B-hGH expression. The lack of mCCPI expression in three of six CD4+ clones, with the expression of CSP-B-hGH in all clones, is in agreement with the results obtained in the CD4 and CD8 depletion studies.

DISCUSSION

T-lymphocyte activation plays an important role in the coordination of the immune response. Although a number of genes are known to be expressed only in activated T-cells, the events regulating inducible T-cell-specific expression are not yet understood. We have begun to characterize transcriptional regulation at a late stage of T-cell activation, using the CSP-B gene as a model. In the present study, we have examined the patterns of expression of a transgene regulated by the CSP-B promoter and 5'-flanking sequences. Expression of the CSP-B-hGH transgene is targeted to activated T-lymphocytes and is co-expressed with the endogenous mouse CCPI gene in lymph nodes and distal small intestine. The transgene is transcriptionally silent in resting T-cells, but is expressed at high levels following stimulation via either the TCR or the IL-2 receptor. The transgene is expressed in both CD4+ and CD8-enriched T-cell populations and in all CD4+ clones tested.

Two striking observations were made when CD4+ clones were generated from a transgenic mouse. First, some clones with either the Th1 or Th2 phenotypes expressed mCCPI. Although mCCPI mRNA has previously been detected in some Th2 clones, this is the first report to our knowledge of mCCPI expression by a Th1 clone. Although the expression of mCCPI in CD4+ T-cells calls into question the designation of mCCPI and CSP-B/CGL-1 as "cytotoxic T-lymphocyte-specific" genes, CD4+ clones of both Th1 and Th2 subtypes have been shown to possess cytolytic activity in redirected cytotoxicity assays. Second, we observed inappropriate expression of the CSP-B-hGH transgene in CD4+ clones that did not express mCCPI. This migration could due to a lack of negative regulatory sequences located outside the −1170 to +31 region that may be required to silence CSP-B/CGL-1 expression in some CD4+ T-cells. Alternatively, the variable expression of mCCPI in mouse T-cell subsets may not be conserved in human T-cells. Although CD4+ T-cell subsets similar to those described in the mouse have been identified in human T-lymphocyte clones, no examination of CSP-B/CGL-1 expression in these cells has yet been published.

We have recently determined that the region necessary and sufficient for CSP-B promoter activation in PEER cells contains AP-1 and cAMP response element consensus sequences. Since transcription factors that bind with these sequences (such as c-fos, c-jun, and CREB) are not T-cell restricted, the tissue specificity of CSP-B expression may be due to other regulatory elements. For example, CSP-B and the other genes of the serine protease gene cluster may be regulated by sequences within a domain organizer like the one found upstream from the human β-globin gene cluster (44). These sequences, termed the locus control region, direct high level, copy number-dependent, integration site-independent expression of linked globin genes in transgenic mice. Since the CSP-B-hGH transgene is sensitive to the flanking sequences surrounding each integration site and since it is inappropriately expressed in some CD4+ cells, we suspect that additional regulatory elements will be required for completely accurate transgene expression.

Only a limited number of T-lymphocyte-specific genes have been analyzed in transgenic systems thus far. Examples include the T-cell receptor genes, the Thy-1 gene, and the CD2 gene, all of which are constitutively expressed in T-cells throughout their development (45-47), and the IL-2 gene, which is rapidly induced in T-cells following activation (9). Several investigators have identified cis-acting sequences in the IL-2 promoter and 5'-flanking region that mediate inducible expression in transfected cell lines (9). One of these, the NFAT-1 motif, has been tested for function in a transgenic system (48). Three copies of the NFAT-1 site were linked to a minimal IL-2 promoter driving the SV40 T-antigen gene. T-antigen expression was found to be inducible in T-lymphocytes in response to 12-O-tetradecanoylphloroglucinol-13-acetate + ionomycin. Low levels of transgene expression were also detected in unstimulated lymphocytes, in 12-O-tetradecanoylphloroglucinol-13-acetate + ionomycin-treated B-lymphocyte populations, and in the dermis. These results suggest that additional sequences are required for T-cell restricted expression of the IL-2 promoter.

Low levels of mCCPI and CSP-B-hGH mRNA were detected in the distal small intestine. The vast majority of T-lymphocytes in murine intestinal epithelia express the alternative γδ T-cell receptor (35). These intraepithelial lymphocytes exhibit constitutive cytotoxicity, presumably reflecting activation by orally ingested antigens (35, 49); these cells therefore provide a model for T-cell activation in vivo. The level of CSP-B-hGH mRNA is highest in the distal small intestine, correlating precisely with the level of mCCPI

2 R. D. Hanson and T. J. Ley, unpublished data.
mRNA (data not shown). Therefore, activation of transgene expression appears to occur under physiologic conditions, further supporting the utility of the CSP-B 5'-flanking region as an activation marker and targeting element.

The expression of the CSP-B-hGH transgene in all activated T-cells should prove useful in future studies involving the manipulation of T-cells in the immune response. The CSP-B/CGL-1 regulatory unit can now be used to specifically target the expression of additional novel gene products to activated T-cells. We are also identifying the DNA sequences between positions -1170 and +31 of the CSP-B/CGL-1 gene that target expression to activated T-cells.

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