JOURNAL OF VIROLOGY, Jan. 1997, p. 771–777 Vol. 71, No. 1
0022-538X/97/$04.00+0

STAT1 Pathway Is Involved in Activation of Caprine Arthritis-Encephalitis Virus Long Terminal Repeat in Monocytes

TIINA SEPP AND SANDRA E. TONG-STARKSEN*

Department of Medicine, University of California-San Francisco and Veterans Administration Medical Center, San Francisco, California 94121

Received 10 July 1996/Accepted 7 October 1996

The caprine arthritis-encephalitis virus (CAEV) long terminal repeat (LTR) is activated by gamma interferon (IFN-γ) in promonocytic cells. We have previously shown that a 70-bp element is necessary and sufficient for the response of the CAEV LTR to this cytokine. At the 5′ end, this 70-bp IFN-γ response element contains sequence similarity to the gamma activated site (GAS). Here we demonstrate that the putative GAS element in the CAEV LTR binds specifically to a cellular factor induced by IFN-γ in promonocytic cells. Substitution mutations in this consensus sequence eliminate binding of the inducible factor. The GAS element from the 70-bp motif is sufficient to confer responsiveness to IFN-γ using a heterologous minimal promoter. Consistent with the binding data, the same mutations in the GAS element eliminate responsiveness to IFN-γ in the context of both a functional CAEV LTR and a heterologous promoter. The cellular factor that binds to the GAS element is present from 5 min to 14 h after stimulation with IFN-γ. Binding of the nuclear factor to the GAS element in the CAEV LTR is inhibited by antibody directed against STAT1 (p91/84). Thus, the GAS sequence in the CAEV LTR is essential for the response to IFN-γ and a STAT1-like factor binds to this site. The STAT-1 signaling pathway provides at least one mechanism for activation of the CAEV LTR by IFN-γ in monocytes. These data are the first demonstration of a role for a STAT family member in the regulation of a viral promoter.

Caprine arthritis-encephalitis virus (CAEV) causes a symmetric, peripheral arthritis in infected adult goats (3). CAEV belongs to the group of nononcogenic retroviruses called lentiviruses. Other members of the group include human immunodeficiency virus type 1 (HIV-1), HIV-2, simian immunodeficiency virus, equine infectious anemia virus, feline T-lymphotropic virus, and visna virus (11). All members of this retrovirus group are species specific, have tropism for the cells of the monocyte-macrophage lineage and are characterized by persistent infections in vivo. Although production of the virus is restricted in infected monocytes, differentiation into macrophages results in increased viral expression and an associated inflammatory response. In vivo expression and replication of the virus in tissue macrophages correlates with development of clinical disease, such as inflammatory arthritis (11, 17).

Several cellular signalling pathways have been shown to be involved in the regulation of lentiviral transcription in monocytes. The HIV-1 long terminal repeat (LTR) is regulated in monocytes by nuclear factor NF-κB (9). Members of the *ets* family of proto-oncogenes, as well as NF-κB, are important for increased expression of the HIV-2 LTR during monocyte differentiation (15). The FOS-JUN heterodimer binds to a site for activator protein (AP-1) and is involved in transcriptional activation of the visna virus LTR (24). The factors required for activation of the CAEV LTR have not been characterized.

Another pathway that leads to differentiation of monocytes is initiated by gamma interferon (IFN-γ). This cytokine is secreted by an activated subpopulation of T cells (Th1) and natural killer cells during inflammatory responses to viral infection (4). Signalling through the IFN-γ receptor leads to activation of a distinct pair of tyrosine kinases, JAK1 and JAK2, which belong to the Janus kinase family (22). However, recent studies have demonstrated an additional requirement for a serine kinase and serine phosphorylation for maximal activation of transcription (29). The activated kinases lead to phosphorylation of a latent cytoplasmic protein, STAT1 (signal transducer and activator of transcription). Dimeric phosphorylated STAT1 translocates to the nucleus, binds to the gamma activation site (GAS) consensus element in IFN-γ-responsive promoters, and activates IFN-γ-dependent transcription of different cellular genes (5, 22, 25).

We have demonstrated in a previous report that the CAEV LTR is activated by stimulation of monocytes with IFN-γ. A 70-bp region is necessary and sufficient for the IFN-γ response in the promonocytic cell line U937 (26), and we call this region the CAEV 70-bp interferon response element (70-bp IRE). A variety of agents, including IFN-γ, can be used to induce differentiation of these cells into a more mature phenotype (13, 19, 20). Differentiation of U937 cells induced by IFN-γ is associated with the binding of two distinct cellular factors to the GAS sequence: IFN-γ activation factor (STAT1 or GAF) and differentiation-induced factor (DIF) (7). The 5′ end of the CAEV 70-bp IRE contains sequence similarity to the GAS consensus element. We characterized the binding of cellular factors to and the function of this putative GAS element. Here we demonstrate that a GAS consensus site in the 70-bp IRE is necessary for regulation of the CAEV LTR by IFN-γ and is sufficient to confer IFN-γ responsiveness. We further identify the cellular factor binding to this sequence as STAT1. To our knowledge, these findings are the first demonstration of regulation of a viral promoter by a STAT family member.

The LTRs of two different isolates of CAEV, p1244 and Co, contain one or two copies of the 70-bp IRE, respectively (Fig. 1). Examination of these LTRs for sequence similarities to binding sites for known transcription factors revealed that the

* Corresponding author. Mailing address: Department of Medicine, UCSF Immunology/Arthritis Section (111R), VAMC, 4150 Clement St., San Francisco, CA 94121. Phone: (415) 750-2104. Fax: (415) 750-6920. E-mail: TONG SANDRA_@SANFRANCISCO.VA.GOV.

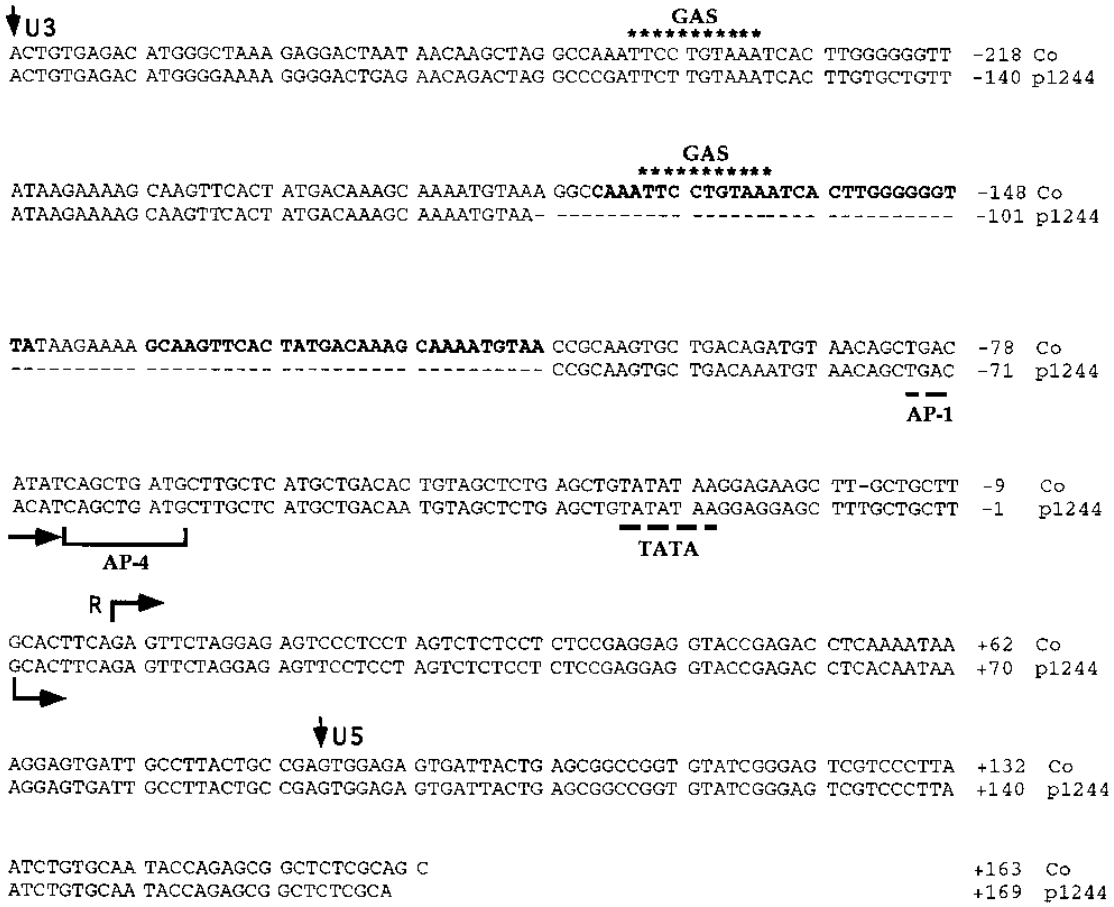

FIG. 1. Sequences of the CAEV LTR. The published sequences of two different CAEV strains, Co and p1244, are shown (14, 23). Nucleotide positions are numbered relative to the start sites of transcription initiation, which are indicated with bent arrows. Vertical arrows show the boundaries of the U3, R, and U5 regions. Putative binding sites for AP-1, AP-4, and GAS are shown. The TATA box is underlined. Dashes in strain p1244 indicate the absence of a 70-bp sequence motif that is duplicated in strain Co. The 5′ and 3′ regions of the CAEV 70-bp IRE are in boldface.

70-bp IRE contains an imperfect palindrome at the 5′ end with sequence similarity to the canonical GAS consensus element that has been described in the promoters of several cellular genes regulated by IFN-γ (5).

To identify the cellular factors interacting with the 70-bp IRE in the CAEV LTR, we synthesized double-stranded DNA oligonucleotides corresponding to the 5′ (positions −175 to −146) and the 3′ (positions −137 to −108) regions of the CAEV 70-bp IRE (Fig. 1 and 2A). The 5′ region contains sequence similarity to the GAS consensus element, while the 3′ region is divergent. We performed electrophoretic mobility shift assays (EMSAs) with nuclear extracts from the promonocytic cell line U937, which undergoes partial differentiation when treated with IFN-γ. As shown in Fig. 2B, nuclear factors induced by IFN-γ bound to the radiolabeled double-stranded oligonucleotide containing the 5′ region of the CAEV 70-bp IRE (lane 2). These factors were not present in unstimulated U937 cells (Fig. 2B, lane 1). Factors induced by IFN-γ also bound to the palindromic IRE (pIRE) (Fig. 2A) from the human intercellular adhesion molecule 1 (ICAM-1) promoter (2) used as a positive control (Fig. 2B, lane 3). The protein complexes (Fig. 2B, lanes 2 and 3) appeared to have different mobilities. This difference in mobility might be due to the binding of different but antigenically related factors to these consensus sequences. Alternatively, the different mobilities might be due to the different lengths of the oligonucleotide probes used for the EMSAs (23 and 34 nucleotides, respectively; see Fig. 2A), since the unbound radiolabeled ICAM-1 pIRE and the CAEV 5′ IRE migrated with different mobilities in nondenaturing gels (data not shown).

To demonstrate the role of the putative GAS element in binding of this factor, we disrupted the imperfect palindrome in the 5′ 70-bp IRE by mutating 2 bp (Fig. 2A, m5′ 70-bp IRE). These substitution mutations eliminated the binding of factors induced by IFN-γ to the 5′ 70-bp IRE (Fig. 2B, lane 4).

The nuclear factors induced by IFN-γ did not react with the 3′ 70-bp IRE (Fig. 2B, lane 5), which does not have homology to the GAS consensus element. An additional complex, observed with radiolabeled oligonucleotides, was present in both stimulated and unstimulated cell lysates. The addition of nonspecific competitor DNA decreased the amount of this complex significantly (data not shown), and thus, we conclude that this additional complex most likely represented nonspecific DNA-protein interactions. Consequently, a nuclear factor(s) induced by IFN-γ bound to the GAS element in the 5′ 70-bp IRE from the CAEV LTR and to the ICAM-1 pIRE. These factors reacted with neither the mutated GAS oligonucleotide nor the divergent 3′ 70-bp IRE.

The specificity of the complexes induced by IFN-γ was demonstrated in competition experiments. Unlabeled oligonucleotides containing the 5′ 70-bp IRE, the m5′ 70-bp IRE, the ICAM pIRE, or the 3′ 70-bp IRE were added in 30-fold excess

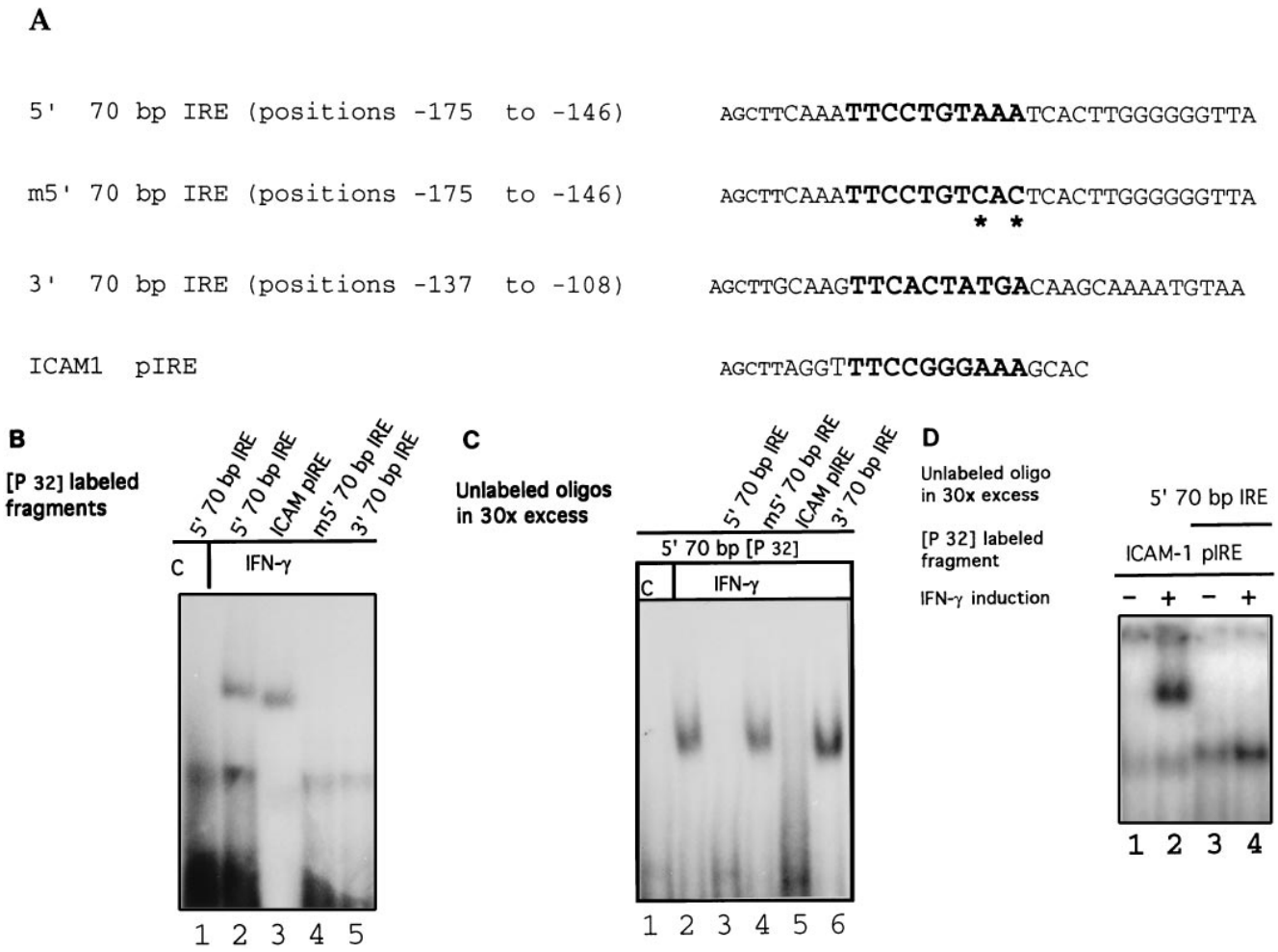

FIG. 2. (A) Sequences of the oligonucleotides used in EMSAs. Sequences of the coding strands of duplex oligonucleotides synthesized for the EMSAs in B and C are shown. GAS consensus sequences are in boldface. *Hin*dIII sites at the 5′ end enable cloning into appropriate vectors. The 2-bp mutations in the GAS element are shown with asterisks. (B) IFN-γ induces a nuclear factor in promonocytic cells that binds to the CAEV GAS. U937 cells were treated with medium alone (lane 1) or with IFN-γ (200 U/ml) (lanes 2 to 5) for 30 min. Nuclear extracts from these cells were incubated with radiolabeled duplex oligonucleotides corresponding to the 5′ (lanes 1 and 2) and 3′ (lane 5) regions of the CAEV 70-bp IRE (5′ 70-bp IRE and 3′ 70 bp IRE, respectively) and a mutant 5′ 70-bp oligonucleotide containing two mutations in the CAEV GAS element (m5′ 70 bp IRE, in lane 4). As a positive control, a pIRE of the ICAM-1 promoter (ICAM pIRE, lane 3) was used. The complexes were resolved by electrophoresis on a 5% native polyacrylamide gel as previously described (2, 7). (C) Specificity of binding demonstrated by competition analyses using unlabeled duplex oligonucleotides. The 5′ 70-bp IRE was radiolabeled and incubated with nuclear extracts from unstimulated U937 cells (lane 1) and from U937 cells stimulated with IFN-γ (lane 2 to 6). Unlabeled oligonucleotides representing the 5′ 70-bp IRE (lane 3), the m5′ 70-bp IRE (lane 4), the ICAM pIRE (lane 5), and the 3′ 70-bp IRE (lane 6) were added in 30-fold molar excess prior to the binding reaction. Complexes were resolved as described above. (D) The similarity of the complexes that bind to the ICAM-1 pIRE and the CAEV 5′ 70-bp IRE was demonstrated by competition analyses. The ICAM-1 pIRE was radiolabeled and incubated with nuclear extracts from unstimulated U937 cells (lanes 1 and 3) and from the cells stimulated with IFN-γ (lanes 2 and 4). The unlabeled 5′ 70-bp IRE (lane 3) was added in 30-fold molar excess prior to the binding reaction, and complexes were resolved as described above.

as specific competitors for binding of nuclear factors to the radiolabeled 5′ 70-bp IRE (Fig. 2C). An excess of the unlabeled 5′ 70-bp IRE, as well as the ICAM-1 pIRE inhibited formation of the complexes induced by IFN-γ (Fig. 2C, lanes 3 and 5, respectively) in a specific manner. Consistent with our hypothesis, competition with an excess of the m5′ 70-bp IRE and the 3′ 70-bp IRE (Fig. 2C, lanes 4 and 6) did not inhibit the binding. To find out if there are similarities between complexes that bind to the ICAM-1 pIRE and the CAEV GAS, we additionally performed competition experiments with the unlabeled CAEV 5′ IRE and the radiolabeled ICAM-1 pIRE. The promonocytic cells were incubated with medium alone (Fig. 2D, lanes 1 and 3) or induced with IFN-γ (Fig. 2D, lanes 2 and 4). The nuclear extracts of these cells were incubated with the radiolabeled ICAM-1 IRE alone (Fig. 2D, lanes 1 and 2) or with the unlabeled CAEV 5′ IRE (Fig. 2D, lanes 3 and 4). The results demonstrate that the unlabeled CAEV 5′ IRE also competes with nuclear factor binding to the ICAM-1 pIRE (Fig. 2D, lane 4). Thus, in promonocytic cells, IFN-γ induces a nuclear factor that binds in a specific manner to the GAS element in the CAEV LTR and this factor is similar to the factor that binds to the cellular ICAM-1 pIRE.

The 70-bp IRE from the CAEV LTR can confer responsiveness to IFN-γ on a heterologous promoter, the herpes simplex virus thymidine kinase (*tk*) promoter, as demonstrated by transient transfections of U937 cells (26). To determine whether the 5′ and/or 3′ region of the 70-bp IRE motif could confer responsiveness to the *tk* promoter, we constructed plasmids containing the 5′ 70-bp IRE [pCAEV(−175/−146)tkCAT], the m5′ 70-bp IRE [pCAEVm(−175/−146)tkCAT], and the 3′ 70-bp IRE [pCAEV(−137/−108)tkCAT], cloned upstream from the *tk* promoter and the bacterial chloramphenicol acetyl-

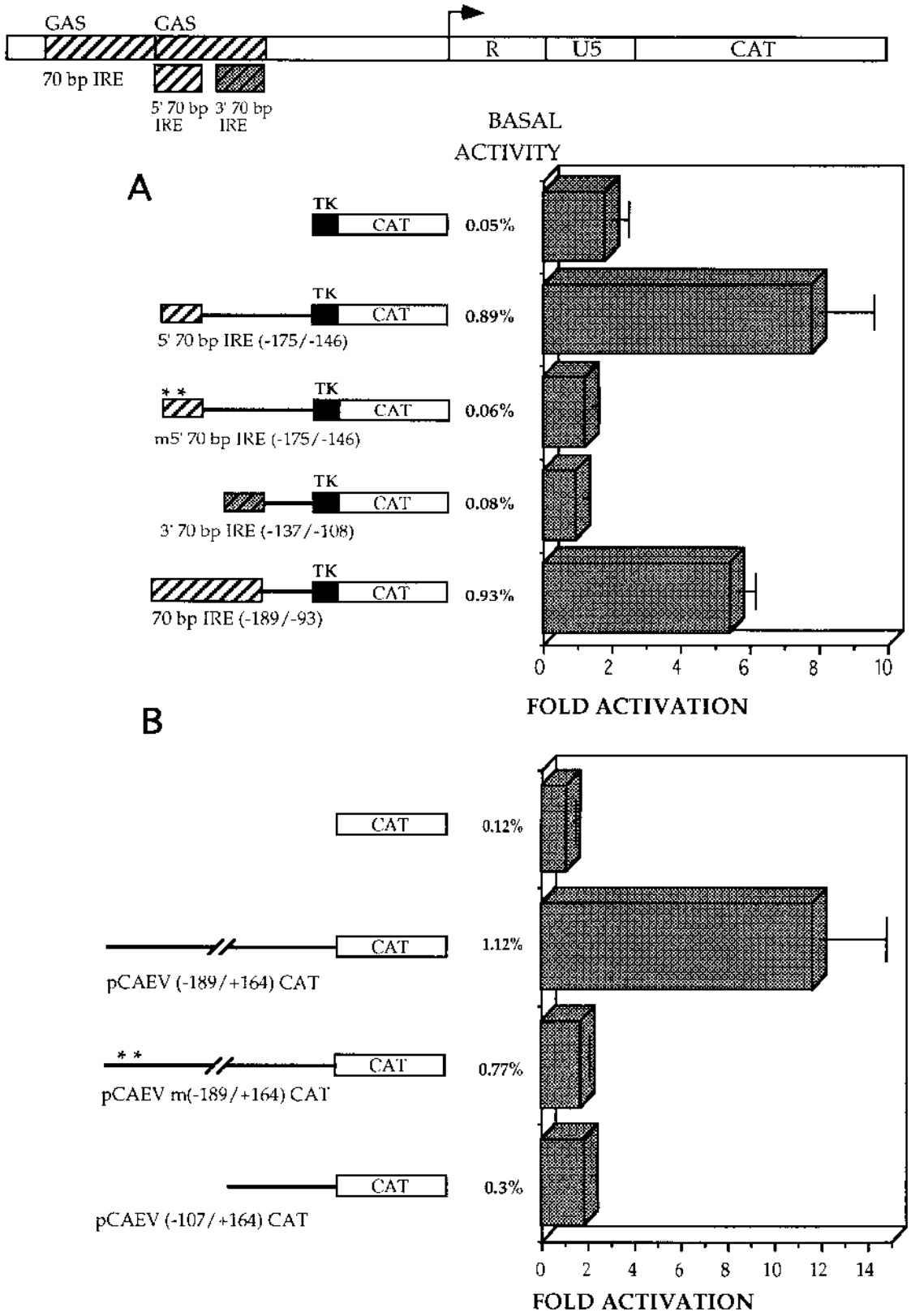

FIG. 3. The GAS sequence in the CAEV LTR is required for IFN response. A schematic diagram of the CAEV LTR is shown at the top. Duplicated 70-bp IREs are indicated by striped boxes. U937 cells were transiently transfected by the DEAE-dextran method with plasmids containing the indicated regions of the CAEV LTR upstream from the herpes simplex virus *tk* minimal promoter (A) or from the CAT reporter gene (B). Transfected cells were stimulated with medium alone (basal) or with IFN-γ (200 U/ml) for 24 h prior to harvesting. Cells were harvested, and basal activity was calculated as previously described (26). Fold activation was calculated as the ratio of CAT activity from stimulated cells over CAT activity from unstimulated cells. Data are the averages of the means from at least three independent transfections.

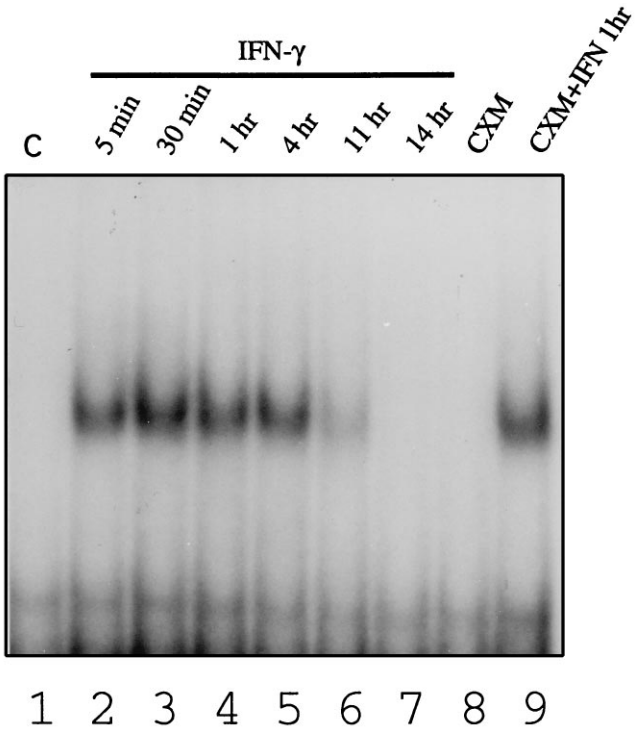

FIG. 4. Time course of activation of the nuclear factor that binds to the CAEV GAS. U937 cells were treated with medium alone (lane 1) or with IFN-γ (lanes 2 to 7) for the indicated periods of time. The cells were treated with cycloheximide (CXM) at 10 μg/ml alone (lane 8) or prior to IFN-γ treatment (lane 9). Nuclear extracts were incubated with the radiolabeled 5′ 70-bp IRE of CAEV (wild-type GAS) and analyzed by EMSA.

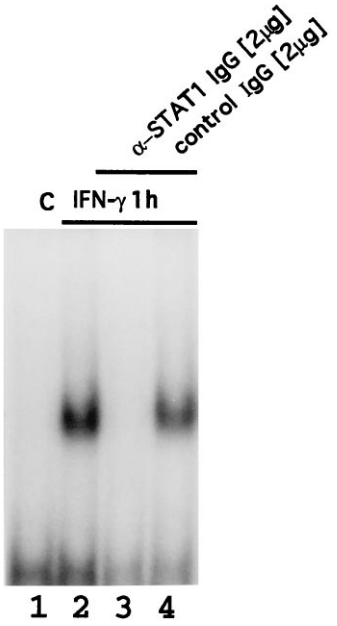

FIG. 5. Inhibition of binding of a nuclear factor to the CAEV GAS by an anti-STAT1 antibody (Santa Cruz Biochemicals). Nuclear extracts from U937 cells treated with medium alone (lane 1) or with IFN-γ (lanes 2 to 4) were incubated with the radiolabeled CAEV 5′ 70-bp IRE. The reaction mixtures were incubated with the anti-STAT1 antibody (lane 3) or with an unrelated control antibody (lane 4) for 20 min at 4°C prior to the addition of the radiolabeled duplex oligonucleotide.

transferase (CAT) reporter gene (Fig. 3A and B). These plasmids were transiently transfected into U937 cells by the DEAE-dextran method (21) and stimulated after 24 h in culture with IFN-γ (final concentration, 200 U/ml) as described previously (26).

The full-length 70-bp IRE and the 5′ 70-bp IRE resulted in increased basal activity and in similar responses to IFN-γ, i.e., 5.3- and 7.8-fold activation, respectively (Fig. 3A). Since comparable levels of activation were observed with the ICAM-1 pIRE (2), the 70-bp IRE, and the 5′ 70-bp IRE were considered sufficient to confer responsiveness to IFN-γ. In contrast, the basal activities and the responses of the m5′ 70-bp IRE, containing the mutated GAS element, and of the 3′ 70-bp IRE were comparable to that of the unresponsive *tk* promoter alone (Fig. 3A). Thus, the lack of responsiveness of the m5′ 70-bp IRE demonstrates the requirement for the GAS sequences for functional activity.

To demonstrate that the GAS element is necessary for the response of the CAEV LTR to IFN-γ, we used a functional CAEV LTR containing one copy of the 70-bp IRE in the plasmid pCAEV(−189/+164)CAT. This plasmid responds to IFN-γ with the same efficiency as the wild-type LTR of strain Co (26), positions −287 to +164 (Fig. 1). The 2-bp substitution mutations described above (Fig. 2A) in the GAS element were introduced into pCAEV(−189/+164)CAT by using in vitro mutagenesis (Altered Sites; Promega). Transient transfections of the functional LTR, pCAEV(−189/+164)CAT, into U937 cells resulted in 11.6-fold activation in the presence of IFN-γ (Fig. 3B). The mutated GAS element pCAEVm(−189/+164)CAT partially decreased the basal activity of the CAEV promoter and eliminated the response to IFN-γ (Fig. 3B). As a negative control, deletion of the 70-bp IRE in the construct pCAEV(−107/+164)CAT also resulted in the loss of IFN-γ responsiveness. Thus, the GAS sequence in the CAEV LTR required for the binding of cellular factors induced by IFN-γ is also essential for its function as an IRE in the context of the CAEV LTR.

To determine the time course of the inducible factor, EMSAs were performed with nuclear extracts from U937 cells treated with IFN-γ for different periods of time. The nuclear factor that bound to the CAEV GAS element appeared at 5 min (Fig. 4, lane 2), was still present at 11 h (Fig. 4, lane 6), and disappeared at 14 h postinduction (Fig. 4, lane 7). Formation of the activation complex did not require de novo protein synthesis, as shown by cycloheximide treatment prior to IFN-γ induction (Fig. 4, lanes 8 and 9).

TABLE 1. Functional GAS elements

Gene	Species	Element
GBP	Human	ATTACTCTAAA
FcgRI	Human	TTTCCCAGAAA
ICSBP	Human	TTTCTCGGAAA
IRF-1	Human	TTTCCCCGAAA
IRF-1	Mouse	TTTCCCCGAAA
c-fos	Human	GTTCCCGTCAA
IFP-53	Human	ATTCTCAGAAA
Ly6E	Mouse	ATTCCTGTAAG
CDC23b	Human	TACCTGAGAAA
IgHe	Mouse	TTCACATGAAC
ICAM-1	Human	TTTCCCGGAAA
CAEV LTR	Goat	ATTCCTGTAAA
Consensus		NTTNCNNNAA

The final identification of the cellular factor that binds to the GAS element in the CAEV LTR was provided by antibody treatment of cell lysates prior to EMSA. Preincubation of U937 nuclear extracts with an anti-STAT1 polyclonal antibody (Santa Cruz Biochemicals, Santa Cruz, Calif.) inhibited binding of nuclear factors to the CAEV element (Fig. 5, lane 3). Preincubation with a nonspecific control antibody had no effect (Fig. 5, lane 5), demonstrating that the inhibition was STAT1 specific. The STAT1 antibody also inhibited complex formation between the factor induced by IFN-γ and the radiolabeled ICAM-1 pIRE but had no effect on the binding of an unrelated protein complex to the radiolabeled AP1 oligonucleotide (data not shown).

Our experiments show that (i) the GAS element in the CAEV LTR is essential for the IFN-γ response and (ii) the nuclear factor that interacts with CAEV GAS is STAT1 or a STAT1-like factor. STAT1 belongs to the STAT family of proteins, which are activated in response to a large number of cytokines, growth factors, and interferons (16). Treatment of monocytes with IFN-γ induces the aggregation of the receptor complex. The recruitment of Janus kinases JAK1 and JAK2 results in the reversible phosphorylation of the IFN-γ receptor α chain, which creates a docking site for the src homology 2 domain of cytoplasmic latent STAT1 (8). STAT1 becomes tyrosine phosphorylated, dissociates from the receptor, dimerizes, and translocates to the nucleus. In the nucleus, phosphorylated STAT1 binds to the GAS consensus element in the promoters of IFN-inducible genes and initiates transcription. The strict requirement of STAT1 for IFN-γ responsiveness has been recently demonstrated in STAT1-deficient mice (6, 18). The CAEV GAS consensus element resembles the well-conserved GAS sequence of cellular genes (Table 1). Our experiments show that in addition to these cellular genes, IFN-γ can activate the transcription of viral genes through the GAS element.

The anti-STAT1 serum used in our experiments (Fig. 5) is STAT1 specific and was raised against a peptide corresponding to amino acids 688 to 700 in the carboxy domain. It has been used to discriminate between different members of the STAT family, such as STAT3, STAT4, and STAT5 (27). The sequence of the carboxy terminus of STAT1 is unique and is not related to those of the other STATs (7). This serum recognizes two naturally occurring splice variants, STAT1α and STAT1β (p91 and p84, respectively). STAT1α contains 38 additional C-terminal amino acids. Although STAT1β becomes phosphorylated and can bind to DNA, it does not activate transcription after induction with IFN-γ (5, 16).

The time course of the appearance of the CAEV GAS binding factor (Fig. 4) is consistent with that of STAT1 (5). Based on our results demonstrating the requirement of CAEV GAS for transcriptional activation (Fig. 3A and B), we conclude that the CAEV GAS binding protein is STAT1α and that STAT1 is at least one pathway by which the CAEV LTR is activated by IFN-γ.

Another factor that can bind to the GAS consensus element is the differentiation-induced factor DIF (7). DIF and STAT1 are clearly distinct molecules, although both are induced in U937 monocytes in response to IFN-γ treatment. DIF has a slower mobility on EMSA than STAT1, binds to selected GAS elements, and requires de novo protein synthesis (7). Antibodies to p91 (STAT1) abolish the binding of STAT1 to GAS but do not affect the binding of DIF to GAS elements (7). The factor that bound to the CAEV GAS sequence in our experiments was STAT1 and not DIF, since antibodies to STAT1 abolish binding to the CAEV GAS element, and the factor does not require de novo protein synthesis (Fig. 4).

Our demonstration of STAT1 involvement in upregulation of the CAEV LTR provides an interesting insight into the multiple roles of IFN-γ, initially identified due to its antiviral effects. The role of IFN-γ as an antiviral agent is documented by three types of clinical observations: (i) IFN production correlates with natural recovery in many viral infections, (ii) inhibition of IFN production or action enhances the severity of infection, and (iii) IFN treatment protects against viral infection (1). One mechanism for the antiviral effect of IFN-γ is a specific inhibitory effect on viral promoters such as the murine cytomegalovirus (CMV) immediate-early promoter (12). Inhibition of the murine CMV promoter correlates with inhibition of viral replication by IFN-γ in infected fibroblasts. Alternatively, IFN-γ can enhance replication of rat CMV in macrophages (10). In addition, the IFN-γ pathway appears to be required for the development and progression of murine AIDS (28). Thus, effects of IFN-γ on viral replication in vivo are variable.

CAEV infection of immature monocytes results in low levels of viral replication. We propose a model in which subsequent infection of the host with other viruses stimulates the production of IFN-γ by natural killer cells and the Th1 subset of T-helper cells. Stimulation of infected monocytes by IFN-γ activates not only cellular genes but also the CAEV promoter through the STAT1 pathway. Activation of the CAEV promoter results in increased viral replication, which could stimulate additional host immune responses. The host immune and inflammatory responses produce the clinical disease that occurs in CAEV-infected goats. Whether the STAT1 pathway is essential for the viral life cycle and disease development in the infected host will be addressed in future experiments.

We thank Janice Clements and B. Matija Peterlin for plasmid constructions; Daniel Rengstorff and Amador Pagtakhan for technical assistance; and T. S. Benedict Yen, W. Seaman, and B. Matija Peterlin for critical reading of the manuscript.

This work was supported by the National Institutes of Health (NIH P60AR20684 Multipurpose Arthritis and Musculoskeletal Diseases Center grant and AI28735), the VA Merit Review Board, and the Northern California Arthritis Foundation.

REFERENCES

1. **Baron, S., and F. Dianzani.** 1994. The interferons: a biological system with therapeutic potential in viral infections. Antiviral Res. **24:**97–110.
2. **Caldehoven, E., P. Coffer, Y. Juping, A. Van de Stolpe, F. Horn, W. Kruijer, and P. Van der Saag.** 1994. Stimulation of the human intercellular adhesion molecule-1 promoter by interleukin-6 and interferon-γ involves binding of distinct factors to a palindromic response element. J. Biol. Chem. **269:**21146–21154.
3. **Crawford, T. B., D. S. Adams, W. P. Cheevers, and L. C. Cork.** 1980. Chronic arthritis in goats caused by a retrovirus. Science **207:**997–999.
4. **Dalton, D. K., S. Pitts-Meek, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart.** 1993. Multiple defects of immune cell function in mice with disrupted interferon-γ genes. Science **259:**1739–1742.
5. **Darnell, D. K. J., I. M. Kerr, and G. R. Stark.** 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science **264:**1415–1421.
6. **Durbin, J. E., R. Hackenmiller, M. C. Simon, and D. Levy.** 1996. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. Cell **84:**443–450.
7. **Eilers, A., M. Baccarini, F. Horn, H. R. A., C. Schindler, and T. Decker.** 1994. A factor induced by differentiation signals in cells of the macrophage lineage binds to the gamma interferon activation site. Mol. Cell. Biol. **14:**1364–1373.
8. **Greenlund, A. C., M. O. Morales, B. L. Viviano, H. Yan, J. Krolewski, and R. D. Schreiber.** 1995. Stat recruitment by tyrosine-phosphorylated cytokine receptors: an ordered reversible affinity-driven process. Immunity **2:**677–687.
9. **Griffin, G. E., K. Leung, T. M. Folks, S. Kunkel, and G. J. Nabel.** 1989. Activation of HIV gene expression during monocyte differentiation by induction of NF-κB. Nature **339:**70–73.
10. **Haagmans, B. L., P. H. Van der Meide, F. S. Stals, A. J. M. Van den Eertwegh, E. Claassen, C. A. Bruggeman, M. C. Horzinek, and V. E. C. Schijns.** 1994. Suppression of rat cytomegalovirus replication by antibodies against gamma interferon. J. Virol. **68:**2305–2312.

11. **Haase, A. T.** 1986. Pathogenesis of lentivirus infections. Nature **322:**130–136.
12. **Harms, J. S., and G. A. Splitter.** 1995. Interferon-γ inhibits transgene expression driven by SV 40 or CMV promoters but augments expression driven by the mammalian MHC I promoter. Hum. Gene Ther. **6:**1291–1297.
13. **Harris, P., and P. Ralph.** 1985. Human leukemic models of myelomonocytic development: a review of the HL 60 and U937 cell lines. J. Leukocyte Biol. **37:**407–422.
14. **Hess, J. L., J. M. Pyper, and J. E. Clements.** 1986. Nucleotide sequence and transcriptional activity of the caprine arthritis-encephalitis virus long terminal repeat. J. Virol. **60:**385–393.
15. **Hilfinger, J. M., N. Clark, M. Smith, K. Robinson, and D. M. Markovitz.** 1993. Differential regulation of the human immunodeficiency virus type 2 enhancer in monocytes at various stages of differentiation. J. Virol. **67:**4448–4453.
16. **Ihle, J. N.** 1996. STATs: signal transducers and activators of transcription. Cell **84:**331–334.
17. **Lairmore, M. D., G. Y. Akita, H. I. Russell, and J. C. DeMartin.** 1987. Replication and cytopathic effects of ovine lentivirus strains in alveolar macrophages correlate with in vivo pathogenecity. J. Virol. **61:**4038–4042.
18. **Meraz, M. A., J. M. White, K. C. F. Sheehan, E. A. Bach, S. J. Rodig, A. S. Dighe, D. H. Kaplan, D. Campbell, K. Carver-Moore, R. N. Dubois, et al.** 1996. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell **84:**431–442.
19. **Murray, H. W.** 1988. Interferon-gamma, the activated macrophage, and host defense against microbial challenge. Ann. Intern. Med. **108:**595–608.
20. **Prieto, J., A. Eklund, and M. Patarroyo.** 1994. Regulated expression of integrins and other adhesion molecules during differentiation of monocytes into macrophages. Cell. Immunol. **156:**191–211.
21. **Queen, C., and D. Baltimore.** 1983. Immunoglobulin gene transcription is activated by downstream sequence elements. Cell **33:**741–748.
22. **Schindler, C., and J. E. Darnell.** 1995. Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. Annu. Rev. Biochem. **64:**621–651.
23. **Sherman, L., A. Yaniv, H. Lichtman-Pleban, S. R. Tronick, and A. J. Gazit.** 1989. Analysis of regulatory elements of the equine infectious anemia virus and caprine arthritis-encephalitis virus long terminal repeats. Virology **63:** 4925–4931.
24. **Shih, D. S., L. M. Carruth, M. Anderson, and J. E. Clements.** 1992. Involvement of FOS and JUN in the activation of visna virus gene expression in macrophages through an AP-1 site in the viral LTR. Virology **190:**84–91.
25. **Steimle, V., C.-A. Siegrist, A. Mottet, B. Lisowska-Grospierre, and B. Mach.** 1994. Regulation of MHC class II expression by IFN-γ mediated by the transactivator gene CIITA. Science **265:**106–109.
26. **Tong, S. E., T. Sepp, and J. Pagtakhan.** 1996. Activation of caprine arthritis-encephalitis virus long terminal repeat by gamma interferon. J. Virol. **70:** 595–599.
27. **Tsukada, J., W. R. Waterman, Y. Koyama, A. C. Webb, and P. Auron.** 1996. A novel STAT-like factor mediates lipopolysaccharide, interleukin 1 (IL-1), and IL-6 signaling and recognizes a gamma interferon activation site-like element in the *IL1B* gene. Mol. Cell. Biol. **16:**2183–2194.
28. **Uehara, S., H. Yasumichi, F. Numata, M. Makino, L. Howard, T. Mizuochi, and K. Takatsu.** 1994. An IFN-γ dependent pathway plays a critical role in pathogenesis of murine immunodeficiency syndrome induced by LP-BM5 murine leukemia virus. Int. Immunol. **6:**1937–1947.
29. **Wen, Z., Z. Zhong, and J. E. J. Darnell.** 1995. Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. Cell **82:**241–250.