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35. We thank U. Banerjee and K. V. Anderson for comments on the manuscript, J. Margolis and J. Posakony for the *hb* cDNA, H. Jäckle for the *Kr* and *kni* cDNAs, E. Gustavson and T. Kornberg for the *en* cDNA, and D. Krantz for pHT4 DNA. Supported by NIH grant 09948 and NSF grant 10451 to J.A.L. and by Ursula Mandel Fellowships to E.S. and F.P.

28 March 1991; accepted 17 June 1991

# Autocrine or Paracrine Inflammatory Actions of Corticotropin-Releasing Hormone in Vivo

KATIA KARALIS,*† HAJIME SANO, JEFFREY REDWINE, SAM LISTWAK, RONALD L. WILDER, GEORGE P. CHROUSOS†

**Corticotropin-releasing hormone (CRH) functions as a regulator of the hypothalamic-pituitary-adrenal axis and coordinator of the stress response. CRH receptors exist in peripheral sites of the immune system, and CRH promotes several immune functions in vitro. The effect of systemic immunoneutralization of CRH was tested in an experimental model of chemically induced aseptic inflammation in rats. Intraperitoneal administration of rabbit antiserum to CRH caused suppression of both inflammatory exudate volume and cell concentration by approximately 50 to 60 percent. CRH was detected in the inflamed area but not in the systemic circulation. Immunoreactive CRH is therefore produced in peripheral inflammatory sites where, in contrast to its systemic indirect immunosuppressive effects, it acts as an autocrine or paracrine inflammatory cytokine.**

CRH WAS ORIGINALLY ISOLATED from the hypothalamus and identified by and named for its property to stimulate anterior pituitary secretion of adrenocorticotropic hormone (ACTH), the systemic hormone that regulates production of glucocorticoids by the adrenal cortex (*1*). CRH and its receptors are widely distributed in many extrahypothalamic sites of the central nervous system (*2*), and this peptide functions as a coordinator of the stress response (*3*). By activating glucocorticoid and catecholamine secretion, central nervous system CRH participates in the suppressive effects of stress on the immune or inflammatory system (*4*). CRH directly stimulates leukocytes to produce immunoregulatory pro-opiomelanocortin (POMC)–related peptides (β-endorphin, ACTH, and α-melanocyte-stimulating hormone) (*5, 6*) and to secrete interleukin 1 (IL-1) and IL-2 (*6, 7*). CRH also stimulates lymphocyte proliferation, enhances the proliferative response of leukocytes to lectins, and increases the expression of the IL-2 receptor on T lymphocytes (*8*). In addition, CRH-binding sites exist in various subpopulations of leukocytes (*9*), and CRH immunoreactivity and mRNA have been detected in resting subpopulations of human white blood cells (*10*). These data suggest that CRH might have local direct effects on immune or inflammatory processes. We have tested the effect of systemic immunoneutralization of CRH on the size of a quantifiable inflammatory response. Carrageenin, a seaweed polysaccharide, is known to elicit a chemical inflammatory response in rats (*11, 12*), which can be quantified in terms of exudate volume and cell concentration in the inflammatory exudate and is sensitive to dose-dependent suppression by glucocorticoids (*12*).

Male Sprague-Dawley rats were injected intraperitoneally with neutralizing rabbit antiserum to CRH (anti-CRH) 1 hour before they were subcutaneously injected with carrageenin (*13*). Both exudate volume and cell concentrations in the treated area were suppressed by approximately 50 to 60%, when compared with the responses in control rats treated first with normal saline, normal rabbit serum, or rabbit antiserum (anti-TSH) to thyroid-stimulating hormone (TSH) (Fig. 1). The specific suppression of the inflammatory response observed after immunoneutralization of CRH had an opposite effect to that expected from neutralization of CRH in the hypothalamic-hypophyseal portal system. The latter should have resulted in hypoglucocorticoidism, which enhances the inflammatory response (*12, 14*). Instead, the results are compatible with local inflammatory effects of CRH. Direct local administration of anti-CRH into the air pouch simultaneously with carrageenin suppressed the inflammatory response to carrageenin to the same extent as systemic administration of this antiserum (*15*). Neutralizing antiserum (anti-TNFα) to TNFα (tumor necrosis factor–α) also suppressed inflammation (Fig. 1). TNFα is a major autocrine or paracrine inflammatory cytokine, which stimulates inflammation directly and by inducing the secretion of IL-1 and IL-6 (*16*). The effects of anti-CRH and anti-TNFα were not additive, indicating that the two antisera might interfere with a

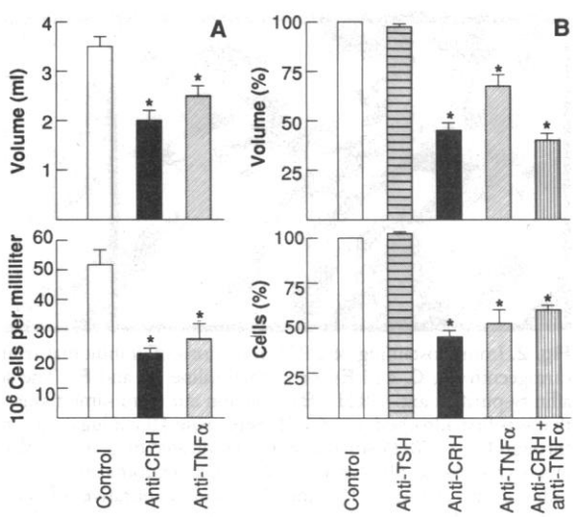


**Fig. 1.** (**A**) Effect of rabbit anti-CRH or anti-TNFα on the inflammatory response of Sprague-Dawley rats to subcutaneous administration of carrageenin (*11, 12*). The mean (± SEM) volume of the inflammatory exudate and concentration of extravasated leukocytes are shown for each treatment group. Rats were injected intraperitoneally with 1 ml of anti-CRH, anti-TNFα, or nonimmune rabbit serum (control) 1 hour before injection of carrageenin. (**B**) Effects of immunoneutralization of CRH and TNFα alone or in combination or treatment with anti-TSH, expressed as a percentage of control values. Data shown are one representative of three separate experiments ($n$ = 9 to 10 animals per group per experiment) in (A) or combined results of three experiments in (B). Asterisks indicate significant differences from control (nonimmune rabbit serum) ($P < 0.05$), determined by analysis of variance.

K. Karalis, J. Redwine, G. P. Chrousos, Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.
H. Sano and R. L. Wilder, Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD 20892.
S. Listwak, Clinical Neuroendocrinology Branch, National Institute of Mental Health, Alcohol, Drug Abuse, and Mental Health Administration, Bethesda, MD 20892.

*Present address: Children's Hospital, Harvard University, Boston, MA 02115.
†To whom correspondence should be addressed.

common aspect of the inflammatory response (Fig. 1).

These results suggested that CRH participates in the inflammatory process in vivo as a local stimulatory agent. Therefore, we examined the site of inflammation for evidence of CRH production. Immunoreactive CRH was detected in the inflamed tissues by immunohistochemistry with an affinity-purified antibody to CRH (Fig. 2) (*17*). The amount of CRH extracted from inflammatory tissue was measured by radioimmunoassay and found to be 400 to 500 pg per gram of wet tissue (*18, 19*). Similar high concentrations of CRH are present in the rat hypothalamus and human placenta, two other sites of CRH production (*19, 20*). The molecular size of CRH acting in inflammatory sites, designated immune CRH, was determined by radioimmunoassay of the fractions (*19*) from high-performance liquid chromatography (HPLC) (*21*). Immune CRH had similar chromatographic mobility to rat or human CRH 1-41, the form produced by the hypothalamus and the human placenta (Fig. 3). There were also immunoreactive bands with lower and higher mobility than CRH 1-41, indicating the presence of precursor (or aggregate) molecules or proteolytic fragments of CRH, respectively.

To examine whether immune CRH produced locally at the inflammatory site might have distant endocrine effects, we measured plasma concentrations of immunoreactive CRH in animals treated with carrageenin or a placebo (*19*). During the 7 hours after treatment, the concentrations of plasma CRH remained low [less than 8 to 10 pg/ml ($\sim 10^{-12}$ M)] in both groups of animals. These concentrations are much lower than that found in the rat hypophyseal portal blood and are not expected to produce biological responses in vivo or in vitro (*22, 23*). In this experimental model, CRH is apparently an autocrine or paracrine, rather than an endocrine, hormone; rapid metabolism, strong binding to local binding proteins (*24*), or both probably prevent CRH from reaching biologically significant circulating concentrations. CRH also has autocrine or paracrine functions when released by chromaffin cells of the sympathetic ganglia or adrenal medulla (*25*) and by Leydig cells of the testes (*26*).

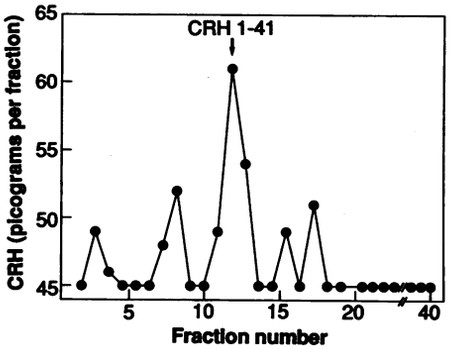


**Fig. 3.** Characterization of immunoreactive CRH extracted from inflamed tissues by HPLC. Extracted peptides were separated by HPLC (*21*), and the amount of CRH in individual fractions was determined by radioimmunoassay (*19*). Synthetic human or rat CRH 1-41 eluted from this column in the fraction indicated.

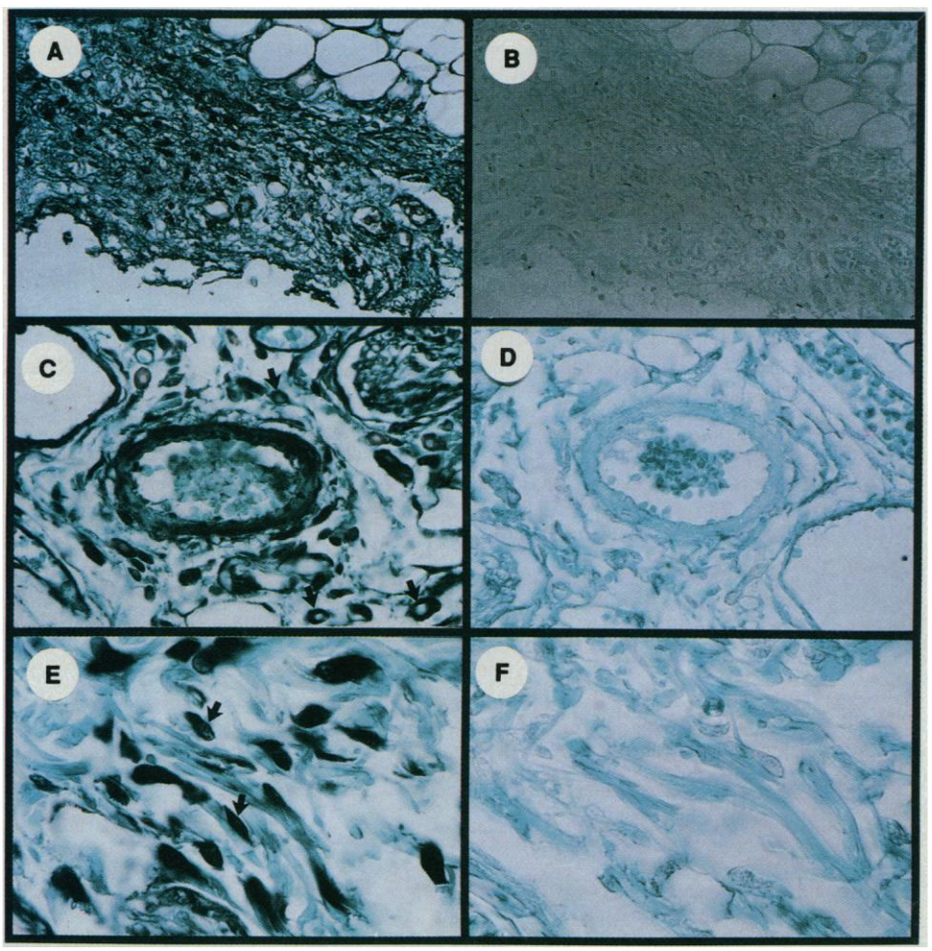


**Fig. 2.** Immunostaining of CRH in sections of granulomas from animals injected subcutaneously with carrageenin (**A**, **C**, and **E**), or normal saline (**D** and **F**). The section shown in (A) was stained with affinity-purified anti-CRH; (**B**) a section cut from same tissue block was stained with anti-CRH IgG that was first adsorbed on a CRH–Sepharose 4B conjugate column. Magnification: (A and B), ×68; (C through F), ×170. Positive staining is indicated by greenish-black deposits. Mononuclear inflammatory leukocytes, tissue fibroblasts, and vascular endothelial cells with intense CRH immunostaining are indicated by arrows in (C) and (E). Semiquantitative analysis of different cell types with a grading system 0 to 4+ gave the following results: mononuclear inflammatory cells 3 to 4+ versus 0 to 0.5+, tissue fibroblasts 3 to 4+ versus 0, and vascular endothelial cells 3 to 4+ versus 0, for inflamed and control tissues, respectively.

The actual position of immune CRH in the inflammatory cascade and the regulation of its secretion cannot yet be discerned. Immune CRH appears to play a role in the initiation or propagation of the inflammatory response in concert with other local factors. Its secretion may be augmented or suppressed by agents that activate or inhibit inflammation, respectively. For example, streptococcal cell wall peptidoglycan-polysaccharide, which induces arthritis in Lewis rats (*27*), also causes the expression of immune CRH in the arthritic joints (*28*). In contrast, somatostatin or glucocorticoids have proportional inhibitory effects on expression of immune CRH and inflammation (*29*). The specific humoral regulators of immune CRH, the presence of local feedback loops, and the extent of POMC-related peptide participation in CRH regulation and actions are not known. However, immune CRH does play a role antithetical to that of hypothalamic CRH, perhaps providing a peripheral counterbalance in the regulation of the inflammatory or immune response.

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13. Characteristics of the anti-CRH (TS 6) antiserum used are as follows: titer, 1:96,000; concentration that causes 10% displacement of radiolabeled tracer ($ED_{90}$), 40 pg/ml; concentration that causes 50% displacement of radiolabeled tracer ($ED_{50}$), 170 pg/ml; cross-reactivity, less than 0.001% for growth hormone–releasing hormone, luteinizing hormone–releasing hormone, antidiuretic hormone, ACTH, luteinizing hormone, follicle-stimulating hormone, TSH, prolactin, β-endorphin, and growth hormone. Characteristics of the anti-TNFα antiserum used are as follows: titer, 1:80,000; $ED_{90}$, 25 pg/ml; $ED_{50}$, 200 pg/ml; cross-reactivity, less than 0.01% for tumor necrosis factor–β.
14. Mean (± SEM) plasma corticosterone was similar 7 hours after carrageenin administration in anti-CRH–treated animals (39 ± 9 μg/dl) and in controls (44 ± 6).
15. Anti-CRH (1.0 ml) was injected synchronously with carrageenin into the air pouch as in Fig. 1.
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18. Approximately 0.5 g of tissue was added to 10 volumes of boiling 2 M acetic acid and kept at 95°C for 10 min. Samples were sonicated for 1 min on ice and centrifuged at 15,000*g* for 30 min. Three volumes of acetone were added to the supernatant. The precipitated proteins were removed by centrifugation (15,000*g*, 30 min), and the supernatant was evaporated to dryness under vacuum. The samples were reconstituted in radioimmunoassay buffer for measurement as described (*19, 23*).
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21. The samples were reconstituted in 200 μl of a solution of 25% acetonitrile, 0.075% trifluoroacetic acid (TFA), and water and injected onto a C18 mBondapak (Waters Associates, Marlboro, MA) analytical HPLC column (3.9 mm by 300 mm). The chromatography was performed with a linear gradient from a 25% acetonitrile, 0.075% TFA, and water solution to an 80% acetonitrile, 0.02% TFA, and water solution over a 30-min period at a flow rate of 1 ml/min. Samples (1 ml) were collected, lyophilized to dryness, reconstituted with radioimmunoassay buffer, and assayed for CRH content as described (*19*).
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29. Dexamethasone (50 μg, Sigma) or somatostatin analog BIM 23014 (10 μg, IPSEN International, Paris, France) was given intraperitoneally to each rat, 1 hour before or simultaneously with carrageenin administration, respectively, as described in (*12*) and Fig. 1.
30. We thank M. Tsokos for helpful discussions.

24 April 1991; accepted 12 August 1991

# Quiescent T Lymphocytes as an Inducible Virus Reservoir in HIV-1 Infection

M. I. BUKRINSKY, T. L. STANWICK, M. P. DEMPSEY, M. STEVENSON*

**To better understand the basis for human immunodeficiency virus type 1 (HIV-1) persistence and latency, the form in which viral DNA exists in the peripheral T lymphocyte reservoir of infected individuals was investigated. In asymptomatic individuals, HIV-1 was harbored predominantly as full-length, unintegrated complementary DNA. These extrachromosomal DNA forms retained the ability to integrate upon T cell activation in vitro. In patients with acquired immunodeficiency syndrome (AIDS), there was an increase in integrated relative to extrachromosomal DNA forms. By analysis of DNA from patient lymphocyte subpopulations depleted of human lymphocyte antigen–Dr receptor–positive cells, quiescent T cells were identified as the source of extrachromosomal HIV-1 DNA. Thus quiescent T lymphocytes may be a major and inducible HIV-1 reservoir in infected individuals.**

THE MAJOR RESERVOIR FOR HIV-1 in the peripheral blood compartment of infected individuals is the $CD4^+$ T lymphocyte (*1, 2*). The high percentage of cells (1 to 0.01%) within this reservoir that contain viral DNA (*2*) is difficult to reconcile with low percentage of infected cells (0.01 to 0.001%) that express viral RNA at levels detectable by in situ hybridization (*3*). In addition, the gradual depletion of $CD4^+$ T lymphocytes during disease progression (*4*) contrasts with the acute cytocidal nature of HIV-1 infection of permissive T lymphocytes in vitro (*5*). These features suggest that a small population of infected cells is permissive for virus replication, whereas the majority of host cells harbor HIV-1 in a minimally replicative yet inducible state.

The life cycle of retroviruses can be separated into pre- and postintegration stages. Integration of HIV-1 DNA with the host cell genome, which depends on the activated state of the host cell (*6*), must occur for a

M. I. Bukrinsky, T. L. Stanwick, M. P. Dempsey, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198.
M. Stevenson, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198, and Division of Virology, National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA.

*To whom correspondence should be addressed.