Anti-HIV Agent Trichosanthin Enhances the Capabilities of Chemokines to Stimulate Chemotaxis and G Protein Activation, and This Is Mediated through Interaction of Trichosanthin and Chemokine Receptors

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Summary

Trichosanthin (TCS), an active protein component isolated from a traditional Chinese medicinal herb Trichosanthes kirilowii, has been shown to inhibit HIV infection and has been applied in clinical treatment of AIDS. The recent development that chemokines and chemokine receptors play important roles in HIV infection led us to investigate the possible functional interaction of TCS with chemokines and their receptors. This study demonstrated that TCS greatly enhanced both RANTES (regulated upon activation, normal T cell expressed and secreted)-- and stromal cell--derived factor (SDF-1α)--stimulated chemotaxis (EC_{50} > 1 nM) in leukocytes (THP-1, Jurkat, and peripheral blood lymphocyte cells) and activation of pertussis toxin–sensitive G proteins (EC_{50} > 20 nM). TCS also significantly augmented chemokine-stimulated activation of chemokine receptors CCR5 and CXCR4 as well as CCR1, CCR2R, CCR3, and CCR4 transiently expressed in HEK293 cells. A mutant TCS with 4,000-fold lower ribosome-inactivating activity showed similar augmentation activity as wild-type TCS. Moreover, flow cytometry demonstrated that the specific association of TCS to the cell membranes required the presence of chemokine receptors, and laser confocal microscopy reveals that TCS was colocalized with chemokine receptors on the membranes. The results from TCS-Sepharose pull-down and TCS and chemokine receptor coimmunoprecipitation and cross-linking experiments demonstrated association of TCS with CCR5. Thus, our data clearly demonstrated that TCS synergizes activities of chemokines to stimulate chemotaxis and G protein activation, and the effects of TCS are likely to be mediated through its interaction with chemokine receptors.

Key words: chemokine receptors • trichosanthin • G proteins • chemotaxis • HIV

Trichosanthin (TCS), a 27-kd protein, is an active component extracted from the root tuber of Chinese medicinal herb Tian-Hua-Fen (Trichosanthes kirilowii) of the Cucurbitaceae family. In the classical Chinese medical reference work Compendium of Materia Medica written in the late 14th century, Tian-Hua-Fen was documented as a drug that resets menstruation and expels retained placentas, and has been used in medical practice in China for hundreds of years. In the early 1970s, TCS was isolated from T. kirilowii and has been used to terminate early and midtrimester pregnancies (1, 2) and to treat ectopic pregnancies, hydatidiform moles, and trophoblastic tumors (2, 3). Pharmacological studies reported that TCS is able to inactivate eukaryotic ribosomes (4, 5) and to suppress the immune responses (6, 7). Moreover, TCS was shown to inhibit HIV replication in infected cells of lymphocyte and mononuclear phagocytic lineage, with no measurable toxicity in uninfected cells (8, 9). In the early 1990s, TCS was applied in the treatment of patients with AIDS or AIDS-related complex in phase I and II studies (10–13). However, the underlying mechanisms of the activities of TCS are not yet well-understood.

Chemokines are a superfamily of small structurally related cytokine molecules characterized by their ability to induce leukocyte migration and related responses (14–18). Chemokines also play important roles in regulation of HIV.
growth, and in angiogenic and developmental processes (14, 19–21). Biological activities of chemokines are mediated by G protein-coupled chemokine receptors (GPCRs) classified as CC or CXC receptors based on the structures and types of chemokines they interact with (14, 15). Of considerable interest is the recent discovery that CC chemokine receptors CCR5, CCR2B, and CCR3 and the CXC chemokine receptor CXCR4 are the essential coreceptors on the cell surface for HIV-1 fusion and infection (22–28). Substantial progress has been made recently in the understanding of chemokine receptor-mediated cellular signaling (29–32). Activation of chemokine receptors by chemokines induces downregulation of chemokine receptors on the cell surface (31, 32). Prevention of HIV-1 infection and inhibition of HIV-1 replication by chemokines, antagonists of chemokine receptors, or mAbs to chemokine receptors, which induce downregulation of chemokine receptors and/or directly block HIV-1 interaction with the coreceptors, have been demonstrated (33–37). Therefore, drugs that target chemokine receptors, the coreceptors of HIV, have great potential in AIDS therapy.

This study was carried out in an attempt to understand the cellular and molecular mechanisms of the activities of TCS against HIV infection. Our results demonstrated that TCS profoundly augments the ability of different chemokines to activate a wide spectrum of chemokine receptors, leading to chemotaxis and G protein activation, and that the effect of TCS is likely to be mediated through its functional interaction with these chemokine receptors.

**Materials and Methods**

**Materials.** Recombinant human RANTES (regulated upon activation, normal T cell expressed and secreted), macrophage inflammatory protein (MIP)-1α, and monocyte chemotactic protein (MCP)-1 were purchased from Sigma Chemical Co., and stromal cell-derived factor (SDF)-1α was from Pharmingen. Native TCS was isolated from T. kiriwii. Recombinant TCS (r-TCS) and a mutant of TCS (m-TCS) were prepared as described previously (38–40). The homogeneity of TCS preparations used was >98%. Rabbit anti-TCS antibodies and an mAb against TCS were provided by Prof. Ming Ye (Shanghai Institute of Cell Biology). Mouse mAb 12CA5 against the influenza hemagglutinin (HA) epitope was obtained from Boehringer Mannheim. [35S]GTPγS and [3H]cAMP were purchased from Amersham Pharmacia Biotech. MEM and RPMI 1640 were from Gibco BRL. GDP and GTPγS were from Sigma Chemical Co. CNBr-activated Sepharose 4B was from Amersham Pharmacia Biotech.

Cloning. CCR5 was cloned as described previously (32). The full-length cDNA encoding CCR1, CCR2B, CCR3, CCR4, and CXCR4 was cloned by reverse transcription PCR and PCR from THP-1 cells (for CCR1, CCR2B, and CXCR4) or PBL cells (for CCR3 and CCR4), using specific primers designed from the published sequences (available from EMBL/GenBank/DDBJ) under accession nos. L09230, U03905, U28694, X58740, and X71635). The amplified human chemokine receptor cDNA fragments were then subcloned into a modified pcDNA3 vector (Invitrogen) with the sequence of the HA epitope tag at the 5′ end of the inserted receptor sequence. The authenticity of the receptor sequences was confirmed by DNA sequencing.
PBS containing 2% BSA at 4°C for 1 h and, after washing with PBS, were incubated with 12CA5 (5 μg/ml) and rabbit TCS-specific antibodies (1:1,000) in PBS containing 2% BSA at 4°C for 1 h. The presence of HA-tagged chemokine receptors and TCS on the cell surface was detected by incubation with FITC-conjugated, affinity-purified goat anti–mouse IgG (Tago) and tetramethyl-rhodamine isothiocyanate (TRITC)-conjugated goat anti–rabbit IgG (Jackson ImmunoResearch Labs). The cells were analyzed on a FACS C alibur™ flow cytometer. Basal cell fluorescence intensity was determined with cells stained with the secondary antibody alone.

Immunofluorescence microscopy. As described previously (48, 49), cells grown on coverslips were fixed in 1% polyformaldehyde for 20 min. After incubation with TCS (100 nM) in PBS containing 2% BSA at 4°C for 1 h and washing twice with cold PBS, cells were treated with 12CA5 mAb and rabbit anti-TCS antibodies. The presence of HA-tagged chemokine receptors and TCS in the cells was then detected with FITC-conjugated, affinity-purified goat anti–mouse IgG and Texas Red-conjugated, affinity-purified goat anti–rabbit IgG (Amersham Pharmacia Biotech), respectively. In addition, control experiments with mock transfection, or in the absence of the first antibodies, or without TCS were performed. Images were recorded using a Leica TCS NT laser confocal scanning microscope.

SDS-PAGE and Silver Staining. The experiment was performed by using a modified silver staining procedure (54). The gel was loaded to 12% SDS-PAGE. The gel was then prefixed with 30% ethanol and 10% acetic acid (HAc) and fixed in 30% ethanol, 0.4 M NaAc, pH 6.0, and 0.03% Na2S2O3. After washing, the gel was incubated in 0.1% AgNO3 and then in 2.5% Na2CO3 with 0.1% Na2S2O3. The reaction was terminated by incubating the gel in 10% HAc.

Immunoprecipitation, Western Blotting, and Cross-linking Experiments. The immunoprecipitation experiment was performed as described (50, 51). HEK293 cells grown in a 60-mm culture dish were lysed in 0.8 ml IP buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% acetic acid, and 0.03% Na2S2O3 containing protease inhibitors) and subjected to 10% SDS-PAGE. The gel was then prefixed with 30% ethanol, 0.4 M NaAc, pH 6.0, and 0.03% Na2S2O3. After washing, the gel was incubated in 0.1% AgNO3 and then in 2.5% Na2CO3 with 0.1% Na2S2O3. The reaction was terminated by incubating the gel in 10% HAc.

Statistical Analysis. Each experimental point was performed in duplicate, and at least three independent experiments were carried out. Data are expressed as means ± SE of all determinations. Statistical significance of the experimental results was obtained by Student’s t test. P < 0.05 was accepted as denoting statistical significance.

Results

TCS Enhances Chemokine-Induced Chemotaxis in THP-1 Cells. Chemotaxis is the prototypic function of chemokines, and thus serves as a biologically relevant functional in vitro assay for chemokine receptor activation (41, 42). THP-1 cells are of human leukocyte origin and express functional CCR1 (52), CCR5 (53), and CXCR4 (Zhao, J., and G. Pei, unpublished observation) and therefore were used in the chemotaxis experiments. THP-1 cells showed a classic bell-shaped chemotactic response upon exposure to increasing concentrations of either RANTES (Fig. 1A) or SDF-1α (Fig. 1B), and both concentration–response curves reached maximum at 1 nM of chemokine (Fig. 1, A and B). The presence of 2 nM TCS alone did not significantly affect chemotaxis (Fig. 1, A–C). However, cotreatment of 2 nM TCS with RANTES or SDF-1α (0.1 nM and above) strongly increased cell migration induced by either chemokine (to ∼300% at chemokine concentrations of 1–10 nM). The concentration–effect curves of TCS on chemokine-induced chemotaxis show that a significant increase of RANTES- and SDF-1α-stimulated chemotaxis occurred.
at TCS concentrations of as low as 0.5 nM, and 2 nM TCS resulted in the maximal enhancement of ~250% (Fig. 1 C).

TCS Enhanced Chemokine-induced G Protein Activation in THP-1 Cells. Our previous study demonstrated that stimulation of chemokine receptors by their agonists activates membrane-associated Gi/Go proteins using [35S]GTPγS binding assay (32). As shown in Fig. 2, A and B, RANTES (agonist of both CCR1 and CCR5) and SDF-1α (agonist of CXCR4) activated membrane-associated G proteins in a concentration-dependent manner in THP-1 cells. TCS (0.2 μM) alone did not have a significant effect on [35S]GTPγS binding. But interestingly, in the presence of 0.2 μM TCS, RANTES- or SDF-1α-stimulated G protein activation increased significantly and the maximal stimulation induced by RANTES and SDF-1α increased by 150 and 200%, respectively (Fig. 2, A and B). As shown in Fig 2 C, the ability of TCS to enhance chemokine RANTES- and SDF-1α-induced G protein activation was dependent on TCS concentration (EC50 = 20 nM). At 5 nM or higher concentration, TCS showed a significant enhancement effect, and in the presence of 200 nM TCS, RANTES- and SDF-1α-induced chemokine receptor stimulation increased by two- to threefold. TCS alone did not have a significant effect on basal [35S]GTPγS binding (Fig. 2 C).

TCS Augmented Chemokine-induced Signaling in PBLs and Jurkat Cells. To test whether TCS can enhance the capability of chemokine to activate chemokine receptors in other cells, PBLs and Jurkat cells were used in this study. As shown in Fig. 3, A and B, TCS significantly enhanced the activation of G proteins induced by RANTES, MCP-1, and SDF-1α in PBLs and by RANTES and SDF-1α in Jurkat cells, respectively. Neither MCP-1 alone nor MCP-1 plus TCS resulted in any stimulation of G protein activation in Jurkat cells that lack CCR2, the receptor of MCP-1. This indicates that the specificity of the effects of TCS relies on both chemokine and chemokine receptor. In the chemotaxis assay, TCS also increased the efficacies of RANTES and MCP-1 to induce cell migration in PBLs (Fig. 3 C) and of RANTES and SDF-1α in Jurkat cells (Fig. 3 D). These data clearly demonstrate that TCS enhances the ability of chemokines to stimulate chemokine receptors and to induce chemotaxis in leukocytes.

The Effects of TCS Were Chemokine Receptor Dependent and Required G Proteins. In HEK293 cells transiently expressing CCR5 (Fig. 4, A and C) or CXCR4 (Fig. 4, B and D), TCS significantly enhanced both RANTES- and SDF-1α-stimulated G protein activation. The effect of TCS in these cells was chemokine concentration and TCS concentration dependent. However, in the mock-transfected HEK293 cells, neither was chemokine-stimulated [35S]GTPγS binding observed nor did TCS show any augmentation effects when used together with RANTES or SDF-1α under the same conditions. In addition, MCP-1, an agonist of CCR2, in either the absence or presence of TCS, was not able to stimulate G protein activation in HEK293 cells transfected with CCR5 (data not shown). Chemokine receptors are able to couple to G1 and Gq proteins. Activation of chemokine receptors cause activation of membrane-associated G proteins and results in inhibition of adenylyl cyclase. As shown in Fig. 5, RANTES and SDF-1α caused inhibition of adenylyl cyclase activity in HEK293 cells transiently expressing CCR5 or CXCR4. Coapplication of TCS under such conditions considerably increased the efficacies of both
chemokines to inhibit cellular cAMP production (Fig. 5). Chemokine-induced inhibition of cAMP production and the enhancement of this by TCS were abolished by pertussis toxin (data not shown). The above results further demonstrate the indispensability of both chemokines and the corresponding chemokine receptors for TCS to exert its effects.

The Effects of TCS Extended to Many Other Chemokine Receptors. To test the potential effects of TCS on cellular signaling mediated by other chemokine receptors and GPCRs in addition to CCR5 and CXCR4, chemokine receptors CCR1, CCR2B, CCR3, and CCR4 and κ and δ opioid receptor were transiently expressed in HEK293 cells. As shown in Fig. 6, TCS significantly enhanced G protein activation mediated by CCR1, CCR2B, CCR3, and CCR4, but failed to enhance opioid agonist–induced G protein activation mediated by either κ (Fig. 6) or δ opioid receptor (data not shown). The above data suggest that in addition to CCR5 and CXCR4, TCS could exert its effect on other members of the chemokine receptor family, probably via a similar mechanism, but the effect of TCS is chemokine receptor specific and may not extend to other peptide Gi/Go-coupled receptors.

The Effects of TCS on Chemokine Receptor Activation Were Independent of Its Ribosome-inactivating Activity. As shown in Fig. 6, after denaturation, TCS lost its ability to enhance chemokine receptor–mediated G protein activation. Furthermore, the enhancement effects of TCS were also blocked by preincubation with the purified mAb against TCS (data not shown). These experiments indicate that TCS is responsible for the observed magnification of chemokine-induced signaling. TCS was originally isolated from *T. kirilowii*, and the recombinant TCS with comparable activities was later successfully produced from *Escherichia coli* (38, 39). As shown in Fig. 7, r-TCS compared with native TCS conferred indistinguishable magnification effects on the chemokine-induced G protein activation and chemotaxis of leukocytes. These data argue that it is the presence of TCS, not any impurities from the preparation, that causes the observed effects on chemokine receptor activation and provides the molecular basis for structure-function studies of TCS.

TCS has been identified as a type I ribosome-inactivating protein (RIP) with a wide spectrum of biological and pharmacological activities. Recent studies showed that mutation at position 120-123 (Lys-Ile-Arg-Glu to Ser-Ala-Gly-Gly) in TCS causes a 4,000-fold decrease in ribosome-inactivating activity (54), implying that this region of the TCS molecule plays a critical role in maintaining its ribosome-inactivation activity. However, this very mutant of TCS (m-TCS) showed similar, or perhaps even higher, enhancement on chemo-
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Figure 6. TCS enhances chemokine receptor–mediated G protein activation. Membranes from THP-1 and HEK293 cells transiently transfected with chemokine receptors CCR1, CCR2B, CCR3, or CCR4, or k-opioid receptor (KOR) were challenged with RANTES, MIP-1β, MCP-1, or SDF-1α in the absence or presence of 0.2 μM TCS or denatured TCS (TCS denatured at 60°C for 20 min) at 30°C for 60 min as indicated. The [35S]GTPγS binding of each sample was then measured as described in Materials and Methods. Data were mean ± SE of three independent experiments performed in duplicate.

Discussion

TCS has been used in the clinical treatment of patients with AIDS or AIDS-related syndromes, but its underlying mechanisms are not well-understood. Recent discoveries that the chemokine receptors CCR5, CXCR4, CCR2B, and CCR3 are HIV-1 coreceptors have thrown new light on the combat against AIDS and other viral diseases. In this work, the effects of TCS on chemokine-stimulated chemotaxis and cellular signaling events and the potential interaction of TCS with the chemokine receptors were investigated. Our results demonstrated that TCS significantly enhanced the chemokine-induced leukocyte chemotaxis, and that the
The effect of TCS was primarily due to its ability to synergize chemokine-dependent activation of chemokine receptors and subsequent receptor-mediated signaling. Our data also revealed the specific association of TCS to the cell membranes with the expression of chemokine receptors and the colocalization and coimmunoprecipitation of TCS with chemokine receptors, suggesting the possibility of direct interaction of TCS with chemokine receptors. Furthermore, the mutant TCS, which lacks the ribosome-inactivating activity, possessed similar enhancement activity as wild-type TCS. Taken together, these results brought to light that TCS was able to functionally interact with a broad spectrum of chemokine receptors as a potent coactivator, which may be one of the mechanisms underlying application of TCS in AIDS treatment.

Although our results demonstrated that TCS functionally interacts with chemokine receptors and is colocalized with the receptors on the cell surface, it remains unclear how TCS is able to costimulate the activation of many different kinds of chemokine receptors by their corresponding chemokines (such as RANTES, SDF-1α, MIP-1β, and MCP-1). One possibility could be that TCS induces the conformational change of chemokine receptors through direct physical contact at the putative binding site that possesses a common structural feature shared by these receptors. Alternatively, TCS may exert its effects through indirect interaction with a third partner on the cell surface, forming a complex capable of interacting with chemokine receptors. Our data sug-

Figure 7. The effects of mutant TCS on chemokine receptor activation and chemotaxis. THP-1 cells were stimulated with SDF-1α (10 nM) in the absence or presence of TCS (isolated from T. kirilowii), r-TCS (recombinant TCS expressed in E. coli), or m-TCS (mutation at position 120–123) (0.2 μM for [35S]GTPγS binding and 2 nM for chemotaxis). [35S]GTPγS binding (A) and chemotaxis (B) were determined as described in Materials and Methods. Data were mean ± SE of at least two independent experiments performed in duplicate. The purities of TCS, r-TCS, and m-TCS were examined using silver staining after SDS-PAGE (C).

Figure 8. Colocalization of TCS with chemokine receptors on cell surface. The HEK293 cells transiently transfected with HA-tagged CCR5 were incubated without (A and B) or with (C and D) 0.1 μM TCS at 4°C for 1 h. The cells were then stained with 12CA5 and FITC-conjugated anti-mouse IgG for transfected CCR5 and with rabbit anti-TCS antibodies and TRITC-conjugated anti-rabbit IgG for TCS. The samples were analyzed by flow cytometry for FITC-labeled (A and C) and TRITC-labeled (B and D) fluorescence signals on the cell surface. Similarly, colocalization of TCS with chemokine receptor on the cell surface was examined by laser confocal fluorescence microscopy (E–J). The cells transiently expressing CCR5 (E–G) or CXCR4 (H–J) were stained with 12CA5/anti-mouse IgG–FITC (for expressed receptor) and anti-TCS/anti-rabbit IgG–Texas Red (for TCS); FITC (green, E and H), Texas Red (red, F and I), and FITC and Texas Red overlapping (yellow, G and J) fluorescent images from the same view were then visualized. Untransfected cells or mock-transfected cells showed negative staining under the same conditions (data not shown).
suggest that on these chemokine receptors, the putative association site(s) at which TCS directly or indirectly interacts appears distinct from the site(s) associated with the chemokines. It has been shown that gp120 envelope glycoproteins of human HIV-1 can physically and functionally interact with chemokine receptors (36, 55-57), and on the receptors the association site(s) of gp120 apparently overlaps with the site(s) associated with the chemokines, since gp120 is able to displace chemokines. Very recent reports from x-ray crystal studies have revealed the structural determinants of gp120 for its binding to CCR5 (58). Similar approaches will be helpful in determining the structural domain of TCS essential for its association with chemokine receptors, since x-ray structural information for TCS is already available (59-61).

Several members of the chemokine receptor family function in association with CD4 to permit entry and infection of HIV-1. CCR5 is a major fusion coreceptor for macrophage-tropic HIV-1 isolates, and CXCR4 is a coreceptor for the entry of T cell line-tropic HIV-1 strains. Chemokines have been shown to inhibit HIV-1 infection, though inefficiently, by interacting with chemokine receptors and thus preventing HIV-1 from using the coreceptors (62, 63). However, the clinical use of excess amounts of chemokines, which induce chemotaxis and activation of leukocytes, may result in undesirable inflammatory side effects. Recently, a CCR5 antagonist from RANTES derivatives has been shown in vitro to block HIV-1 infection of macrophage and lymphocytes at nanomolar concentration (34). Searching for potent antagonists of chemokine receptors is now popularly considered and heavily pursued as one of the most promising strategies for HIV therapy. The result from this study that TCS strongly enhanced the ability of chemokines to activate their receptors may further provide another useful approach to inhibit HIV infection. One of the potential advantages of using coactivators such as TCS could be that the agents, which are not agonists or antagonists, can effectively interact with a wide spectrum of chemokine receptors and may thus promote the efficiency of various endogenous chemokines in blocking HIV infection. It is also worth mentioning that very little about the coactivator(s) of GPCRs has been reported to date. Therefore, the enhancement effects of TCS on chemokine receptor activation may offer a good working model for augmenting our understanding of GPCR activation.

Trichosanthin is a member of the type I RIPs with RNA-glycosidase activity. It has been reported that TCS inhibits HIV replication in vitro in acutely and chronically infected lymphocytes and monocytes (9). Clinical studies also show that TCS treatment may help to prevent loss of CD4+ cells in AIDS patients failing treatment with antiretroviral agents such as zidovudine (10) and even to increase CD4+ cells in other cases (11, 12). It was speculated that the anti-HIV effects of TCS might be due to its ribosome-inactivating activity. However, studies suggest that the mechanism of TCS to inhibit the replication and infection of HIV-1 is different from its activities of ribosome inactivation and immunomodulation (40, 64). In addition, the undesirable side effects of TCS, which have seriously limited its clinical application, may also be related to its activity to induce unwanted cytotoxicities and allergic reactions. Our data in this study showing that TCS greatly enhances activation of chemokine receptors independent of its RIP activity may not only reveal an alternative mechanism underlying the anti-HIV effects of TCS but may also provide a mutagenesis strategy potentially to improve its therapeutic effectiveness and to reduce its side effects.

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References

1. Lui, G., F. Lui, Y. Li, and S. Yu. 1985. A summary of 402 cases of termination of early pregnancy with crystalline preparations of trichosanthen. In Advances in Chinese Medicinal Materials Research. H.-M. Chang, H.-W. Yeung, W.-W. Tso, and A. Koo, editors. World Scientific Publishing Co. Pte., Ltd., Singapore. 327–333.

2. Jin, Y.-C. 1985. Clinical study of trichosanthin. Natl. Acad. Sci. USA. 86:2844–2848.

3. Huang, Y.L. 1987. A clinical study on treatment of malignant diseases and failing antiretroviral agents. Int. J. Pept. Protein Res. 31:265–268.

4. Maraganore, J.M., M. Joseph, and M.C. Bailey. 1987. Purification and characterization of trichosanthen. Homology to the ricin A chain and implications as to mechanism of abortifacient activity. J. Biol. Chem. 262:11628–11633.

5. Yeung, H.-W., W.-W. Li, Z. Feng, L. Barbieri, and F. Sturpe. 1988. Trichosanthen, alpha-momorcharin and betamomorcharin: identity of abortifacient and ribosome-inactivating proteins. Int. J. Pept. Protein Res. 31:265–268.

6. Leung, K.-N., H.-W. Yeung, and S.-O. Leung. 1986. The immunomodulatory and antitumor activities of trichosanthen—an abortifacient protein isolated from ban-hua-fen (Trichosanthes kirilowii). A. J. Allergy Immunol. 4:111–120.

7. Yeung, H.-W., S.P. Poons, T.B. Ng, and W.W. Li. 1987. Isolation and characterization of an immunosuppressive protein from Trichosanthes kirilowii root tubers. Immunopharmacol. 9:25–46.

8. Mc Grath, M.S., S. Santulli, and I. Gaston. 1990. Effects of GLQ223 on HIV replication in human monocyte/macrophage chronically infected in vitro with HIV. AIDS Res. Hum. Retroviruses. 6:1039–1043.

9. Mc Grath, M.S., K.-M. Hwang, S.E. Caldwell, I. Gaston, K.C. Luk, P. Wu, V.L. Ng, S. Crowe, J. Daniels, and J. Marsh. 1989. GLQ223: an inhibitor of human immunodeficiency virus replication in acutely and chronically infected cells of lymphocyte and mononuclear phagocyte lineage. Proc. Natl. Acad. Sci. USA. 86:2844–2848.

10. Byers, V.S., A.S. Levin, A. Malvino, L. Wates, R.A. Robins, and R.W. Baldwin. 1994. A phase II study of effect of addition of trichosanthen to zidovudine in patients with HIV disease and failing antiretroviral agents. AIDS Res. Hum. Retroviruses. 10:413–420.

11. Mayer, R.A., P.A. Sergios, K. Coonan, and L. O’Brien. 1992. Trichosanthen treatment of HIV-induced immune dysregulation. Eur. J. Clin. Invest. 22:113–122.

12. Byers, V.S., A.S. Levin, L.A. Wates, B.A. Starrett, R.A. Mayer, J.A. Clegg, M.R. Price, R.A. Robins, M. Deleaney, and R.W. Baldwin. 1990. A phase II study of trichosanthen treatment of HIV disease. AIDS 4:1189–1196.

13. Kahn, J.O., L.D. Kaplan, J.G. Gambertoglio, D. Breidenstein, C.J. Arri, L. Turin, T. Kibort, R.L. Williams, J.D. Lifson, and P.A. Volberding. 1990. The safety and pharmacokinetics of GLQ223 in subjects with AIDS and AIDS-related complex: a phase I study. AIDS 4:1197–1204.

14. Baggiolini, M. 1998. Chemokines and leukocyte traffic. Nature 392:565–568.

15. Moser, B., M. Roletcher, L. Piali, and P. Loetscher. 1998. Lymphocyte responses to chemokines. Int. Rev. Immunol. 16:323–344.

16. Proost, P., A. Wuyts, and J. van Damme. 1996. The role of chemokines in inflammation. Int. J. Clin. Lab. Res. 26:211–223.

17. Strierer, R.M., P.J. Polverini, D.A. Arenberg, A. Walz, G. Opdenakker, J. van Damme, and S.L. Kunkel. 1995. Role of C-C chemokines as regulators of angiogenesis in lung cancer. J. Leukocyte Biol. 57:752–762.

18. Friedland, J.S. 1996. Chemokines in viral disease. Res. Virol. 147:131–138.

19. Luster, A.D. 1998. Chemokines—chemotactic cytokines that mediate inflammation. N. Engl. J. Med. 338:436–445.

20. Tachibana, K., S. Hotta, H. Iizawa, H. Yoshida, K. Kawa- bata, Y. Kato, Y. Kitamura, K. Matsumura, N. Yoshida, S. Nishikawa, et al. 1998. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature 393:591–594.

21. Zou, Y.R., A.H. Kottmann, M. Kuroda, I. Taniuchi, and D.A. Littman. 1998. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 393:595–599.

22. Furci, L., S. Polo, and P. Lusso. 1998. DBP T lymphocyte-derived chemokines and other HIV-suppressive factors: mini-review. J. Chemother. 10:146–149.

23. Wagner, L., O.O. Yang, E.A. Garcia-Zepeda, Y. Ge, S.A. Kalams, B.D. Walker, M.S. Pasternack, and A.D. Luster. 1998. Beta-chemokines are released from HIV-1-specific cytolytic T-cell granules complexed to proteoglycans. Nature 391:908–911.

24. Littman, D.R. 1998. Chemokines receptors: keys to AIDS pathogenesis? C. el. 93:677–680.

25. Horuk, R. 1998. Chemokines beyond inflammation. Nature 393:524–525.

26. Clapham, P.R., and R.A. Weiss. 1997. Immunodeficiency viruses: spoil for choice of co-receptors. Nature 388:230–231.

27. Thornhill, M.H., and J. Li. 1997. Chemokines and chemokine receptors: the key to understanding AIDS. Oral Dis. 3:3–8.

28. Alkhattib, G., C. Combiadere, C.C. Broder, Y. Feng, P.E. Kennedy, P.M. Murphy, and E.A. Berger. 1996. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 272:1955–1958.

29. Raport, C.J., J. Gosling, V.L. Schweickart, P.W. Gray, and...
37. Amara, A., S.L. Gall, O. Schwartz, J. Salamero, M. Montes, G. Pei. 1998. Chemokine receptor CCR5 functionally couples to inhibitory G proteins and undergoes desensitization. J. Cell. Biol. 71:36–45.

38. Alkhalib, G., M. Locati, P.E. Kennedy, P.M. Murphy, and E.A. Berger. 1997. HIV-1 coreceptor activity of CCR5 and its inhibition by chemokines independent from G protein signaling and importance of coreceptor downmodulation. Virology. 234:340–348.

39. Zhu, R.H., T.B. Ng, H.W. Yeung, and P.C. Shaw. 1992. High level synthesis of biologically active recombinant trichosanthin. Biochem. Biophys. Res. Commun. 186:373–383.

40. Cai, Y.C., G. Pei. 1997. Functional expression, activation and desensitization of opioid receptor-like receptor ORL1 in neuroblastoma NG108-15 hybrid cells. FEBS Lett. 403:91–94.

41. Zhu, X., L. Ding, and G. Pei. 1997. Carboxyl terminus of mitooin is sufficient to confer spore pole localization. J. Cell. Biol. 66:441–449.

42. Cai, Y.C., L. Ding, F. Zhu, X., L. Ding, and G. Pei. 1997. Activation of N-methyl-D-aspartate receptor attenuates acute responsiveness of delta-opioid receptors. Mol. Pharmacol. 51:583–587.

43. Cheng, Z.J., G.H. Fan, J. Zhao, Z. Zhang, Y.L. Wu, L.Z. Jiang, Y. Zhu, G. Pei, and L. M. a. 1997. Endogenous opioid receptor-like receptor in human neuroblastoma SK-N-SH cells activation of inhibitory G protein and homologous desensitization. N. eurotrop. 8:1913–1918.

44. Razanagravo, J.R., and S.R. Nahu. 1995. Modulation by mus-opioid agonists of guanosine-5'-O-3[(3-[35S]thiotri phosphatase binding to membranes from human neuroblastoma SH-SY5Y cells. Mol. Pharmacol. 47:848–854.

45. Cai, Y.C., Z. Zhang, Y.L. Wu, and G. Pei. 1996. Opioid receptor in neuronal cells undergoes acute and homologous desensitization. Biochem. Biophys. Res. Commun. 219:342–347.

46. Cai, Y.C., L. Ma, G.H. Fan, J. Zhao, L.Z. Jiang, and G. Pei. 1997. Activation of N-methyl-D-aspartate receptor attenuates acute responsiveness of delta-opioid receptors. Mol. Pharmacol. 51:583–587.

47. Alkhalib, G., M. Locati, P.E. Kennedy, P.M. Murphy, and E.A. Berger. 1997. HIV-1 coreceptor activity of CCR5 and its inhibition by chemokines independent from G protein signaling and importance of coreceptor downmodulation. Virology. 234:340–348.

48. Zhu, R.H., T.B. Ng, H.W. Yeung, and P.C. Shaw. 1992. High level synthesis of biologically active recombinant trichosanthin. Biochem. Biophys. Res. Commun. 186:373–383.

49. Cai, Y.C., G. Pei. 1997. Functional expression, activation and desensitization of opioid receptor-like receptor ORL1 in neuroblastoma NG108-15 hybrid cells. FEBS Lett. 403:91–94.

50. Zhu, X., L. Ding, and G. Pei. 1997. Carboxyl terminus of mitooin is sufficient to confer spore pole localization. J. Cell. Biol. 66:441–449.

51. Cai, Y.C., L. Ma, G.H. Fan, J. Zhao, L.Z. Jiang, and G. Pei. 1997. Activation of N-methyl-D-aspartate receptor attenuates acute responsiveness of delta-opioid receptors. Mol. Pharmacol. 51:583–587.
tors CXCR4 and CCR5. Proc Natl Acad Sci USA. 95: 8005–8010.

58. Rizzuto, C.D., R. Wyatt, N. Hernandez-Ramos, Y. Sun, P.D. Kwong, W.A. Hendrickson, and J. Sodroski. 1998. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. Science. 280:1949–1953.

59. Pan, K.Z., Z.J. Fu, Y.J. Lin, K.L. Zhou, E. Dodson, Z.W. Chen, and X.M. Ye. 1992. Crystallographic refinement of trichosanthin at 2.6Å resolution. Sci. China B. 35:1203–1213.

60. Pan, K.Z., Y.J. Lin, K.J. Zhou, Z.J. Fu, M.H. Chen, D.R. Huang, and D.H. Huang. 1993. The crystal and molecular structure of trichosanthin at 2.6 Å resolution. Sci. China B. 36:1069–1081.

61. Gao, B., X.Q. Ma, Y.P. Wang, S.Z. Chen, S. Wu, and Y.C. Dong. 1994. Refined structure of trichosanthin at 1.73 Å resolution. Sci. China B. 37:59–73.

62. Cocchi, F., A.L. DeVico, A. Garzino-Demo, S.K. Arya, R.C. Gallo, and P. Lusso. 1995 Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. Science. 270:1811–1815.

63. Paul, W.E. 1995. Can the immune response control HIV infection? Cell. 82:177–182.

64. Pinching, A.J. 1990. Early trials of GLQ223/trichosanthin: what do they show? AIDS. 4:1289–1291.