ence in ability of vagotomized versus nonoperated and sham-operated mice to learn did not account for the difference in CCK-8S to enhance retention.

At the conclusion of the memory experiments, the same group of mice was used to examine the effect of vagotomy on the inhibitory effect of CCK-8S on feeding (*11*). Vagotomy abolished the CCK-8S effect on feeding at low doses, but not at high doses (Fig. 2B). A similar shift in the dose-response curve for the CCK-8S effect on feeding has been shown in vagotomized dogs (*12*).

At the conclusion of the above experiments, on a separate day, the mice were deprived of food for 18 hours and then allowed to feed for 2 hours, after which stomach weights were obtained from 102 mice in the three groups. The mean ratios of stomach weight to body weight were as follows: nonoperated, 1.0 ± 0.04; sham-operated, 1.0 ± 0.04; vagotomized, 2.8 ± 0.5. A one-way ANOVA indicated that operation had a significant effect on the ratios of stomach weight to body weight [$F(2,100) = 10.15$, $P < 0.001$]. Thus, we were able to confirm the effectiveness of the vagotomies.

Our data show that both feeding and peripherally administered CCK-8S enhance memory in mice. This gastrointestinal hormone seems to produce its effect on memory by activating ascending vagal fibers. Further studies are necessary to determine if CCK-8S is responsible for the entire effect of feeding on memory, or, as appears to be the case in the regulation of feeding, if a combination of gastrointestinal hormones act synergistically to produce this effect (*13*). The concentrations of CCK-8S achieved after administration of the optimum memory enhancing dose would be well within the physiological range seen after feeding in rodents (*14*). A link may have evolved between the release of gastric peptides and memory processing in the central nervous system because of the survival advantages for an animal to remember the details of a successful food-foraging expedition.

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9. The nonoperated, sham-operated, and vagotomized mice were obtained from Taconic Farms Inc., Germantown, NY. Operations were performed under the supervision of L. Gunther.
10. Recall score is defined as the percentage of mice in a group making an avoidance on retention test trials 1 to 3 [see (*1*) for details].
11. Animals were deprived of food for 18 hours, and then injected with varying doses of CCK-8S or vehicle (intraperitoneally); their food intake was measured for 1 hour.
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Identification of a T3-Associated γδ T Cell Receptor on Thy-1$^+$ Dendritic Epidermal Cell Lines

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The murine epidermis contains a subpopulation of bone marrow–derived lymphocytes that have a dendritic morphology and that express Thy-1 and T3 cell-surface antigens but not other markers (L3T4 or Lyt-2) characteristic of mature peripheral T lymphocytes. An alternative type of T cell receptor was earlier identified on a subpopulation of murine thymocytes with a similar phenotype (T3$^+$, L3T4$^-$, Lyt-2$^-$), but not on peripheral murine T lymphocytes. Two independently derived Thy-1$^+$, L3T4$^-$, and Lyt-2$^-$ dendritic cell lines of epidermal origin that express a T3-associated disulfide-linked heterodimer composed of a 34-kilodalton γ-chain and 46-kilodalton partner (the δ chain) have now been identified. Analysis of *N*-linked glycosylation revealed that this receptor is similar to that detected on thymocytes. These results demonstrate that Thy-1$^+$ dendritic epidermal cell lines can express γδ T cell receptors in vitro and suggest that Thy-1$^+$ dendritic epidermal cells express such receptors in vivo. The localization of these γδ T cell receptor–expressing cells in the epidermis may be of importance for understanding the function of these receptors.

THE MURINE EPIDERMIS CONTAINS A minor subpopulation of dendritic bone marrow–derived leukocytes that express high levels of the Thy-1 cell-surface antigen but do not express other markers (L3T4 or Lyt-2) characteristic of mature peripheral T lymphocytes (*1*). We recently showed that all Thy-1$^+$ dendritic epidermal cells (DECs) express T3 in situ and thus likely belong to the T lymphocyte lineage (*2*). Analysis of the components of T3-associated T cell receptor (TCR) molecules on Thy-1$^+$ DECs has been facilitated by the availability of several long-term lymphokine-dependent cell lines derived from Thy-1$^+$ DECs that maintain their phenotype in vitro (*1, 2*). We detected a T3-associated 34-kD TCR γ chain on the cell surface of two Thy-1$^+$ DEC cell lines that was apparently disulfide-linked to a 34-kD molecule; this finding suggested that the γ chain might be expressed as a homodimer on these cell lines (*2*). We have now analyzed T3-associated TCR molecules on three additional Thy-1$^+$ DEC cell lines and demonstrate that two lines express a T3-associated disulfide-linked heterodimer composed of 34-kD γ chain and a 46-kD partner (termed the δ chain). These results indicate that Thy-1$^+$ DEC cell lines can express the TCR γ chain as a disulfide-linked component of a heterodimeric TCR and suggest that Thy-1$^+$ DECs express such receptors in vivo and thereby represent one of the major extrathymic T cell populations with this phenotype.

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Three Thy-1$^+$ DEC cell lines (T195, T93, and T245) were used in these studies. All three are T3$^+$, L3T4$^-$, Lyt-2$^-$, Ia$^-$, and express the interleukin-2 (IL-2) receptor, and all three have maintained this phenotype in culture for at least 5 months. Although T195 was originally isolated from purified Thy-1$^+$ DEC cells, it does not express Thy-1, in contrast to T93 and T245. To investigate TCR expression on these cell lines, we surface-labeled them with ^{125}I and lysed them in digitonin lysis buffer (which is known to keep the association between T3 and TCR intact). After preclearing, we carried out precipitations with a control antiserum and the antiserum R9 directed against the T3 δ chain (anti-T3δ) (*3*). These precipitates were analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing and reducing conditions (Fig. 1). Under nonreducing conditions, a T3-associated 80- to 90-kD complex was detected in the anti-T3δ precipitates from these three cell lines, presumably representing the TCR molecules. The 80- to 90-kD complex precipitated from the T195 cell line could be reduced to subunits of approximately 40 kD.

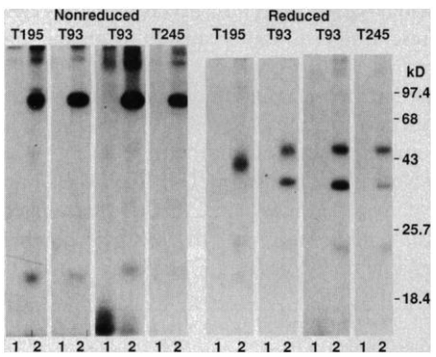

Fig. 1. Analysis of T3-associated TCR molecules on T3$^+$ DEC-derived cell lines T195, T93, and T245 after cell-surface labeling with ^{125}I. In the first experiment, T195 and T93 cells were analyzed, and in the second experiment T93 and T245 were analyzed. All cell lysates were in 1% digitonin, and precipitations were carried out with (lanes 1) control rabbit serum and (lanes 2) R9, a rabbit antiserum specific for T3δ. Precipitates were analyzed on SDS-PAGE gels under reducing or nonreducing conditions as indicated. Cells (10^8) were surface-labeled by lactoperoxidase-catalyzed iodination as described previously (*4, 5*). Cells were lysed in a lysis-wash buffer (1 ml) with 1% digitonin (*14*) for 30 minutes, after which insoluble material was removed by centrifuging at 13,000*g* for 15 minutes. The lysates were cleared with normal rabbit serum and protein A agarose beads (PAA beads) (BRL; Gaithersburg, Maryland), and specific immunoprecipitations were carried out by mixing 100 μl of lysate and 5 μl of antiserum at room temperature for 60 minutes and then incubating for 60 minutes after the addition of 5 μl of PAA beads. The beads were washed four times before being suspended in sample buffer as described by Laemmli (*15*) to elute the immunoprecipitates from the beads. The immunoprecipitates were analyzed by SDS-PAGE and autoradiography.

In contrast, subunits of 34 and 46 kD were detected after reduction of the 80- to 90-kD complex from T93 and T245. These chains resembled the γ chain–containing TCRs expressed on a subset of murine thymocytes (*4, 5*). To investigate this possibility, we labeled T195, T93, and T245 with ^{125}I and lysed the cells in either NP-40 or digitonin lysis buffer. Precipitations with the NP-40 cell lysates were carried out with antiserum to TCR γ (anti-γ) in the absence or presence of the carboxyl-terminal peptide against which the antiserum was raised (this antiserum reacts better in NP-40 cell lysates than in digitonin cell lysates) whereas anti-T3δ precipitations were carried out with the digitonin cell lysates. These precipitates were analyzed on two-dimensional nonreducing and reducing gels (Fig. 2). No anti-γ precipitate was obtained from the T195 cell lysate. However, from both the T93 and T245 cell lines anti-γ precipitated a disulfide-linked heterodimer composed of a 34- and a 46-kD chain (Fig. 2, A and D). The reactivity of the antiserum could be completely blocked by the presence of the γ peptide during the immunoprecipitation procedure (Fig. 2, B and E). A similar complex was obtained in the anti-T3δ precipitations from these cell lines (Fig. 2, C and F).

We next investigated whether anti-γ reacted specifically with the 34-kD chain or the 46-kD chain, or both. Monoclonal antibody to the T3 ε chain (anti-T3ε) was used to carry out precipitations with ^{125}I-labeled digitonin cell lysates of T93 and T245 (*6*). To dissociate the disulfide-linked 34- and 46-kD chains, we reduced these precipitates by adding dithiothreitol (DTT), heated the material at 68°C for 5 minutes, then alkylated with iodoacetamide. The reduced and alkylated material was subsequently divided into two portions, and one of these was used to carry out precipitation with anti-γ. All samples were subsequently analyzed on SDS-PAGE (Fig. 3). In the anti-T3ε precipitate, both the 34- and 46-kD proteins are present (lanes 1 and 3), but after reduction and alkylation of the precipitates, anti-γ precipitated only the 34-kD protein (lanes 2 and 4). These results identify the 34-kD protein as the γ chain and prove that it is associated with T3. However, we cannot exclude the possibility that the 46-kD chain is a product of the Cγ4 locus because the anti-γ is specific for the carboxyl termini of Cγ1, Cγ2, and Cγ3 only (*4, 5*).

We next determined the extent of *N*-linked glycosylation of these TCR chains. Anti-T3δ precipitates of T93 and T245 were either mock-digested or treated with peptide:*N*-glycosidase-F and subsequently analyzed on SDS-PAGE (Fig. 4A). Removal of *N*-linked carbohydrates resulted in the generation of 34- and 31-kD chains from the original 46- and 34-kD chains, respectively. Since this result does not definitively demonstrate which protein backbones correspond to the mature 34- and 46-kD cell surface proteins, the 34- or 46-kD molecules were isolated by electroelution from gel slices. These samples were then either mock-digested or treated with peptide:*N*-glycosidase-F and subsequently analyzed on SDS-PAGE (Fig. 4B). The results show that both the 34- and 46-kD molecules contain *N*-linked glycosyl residues and have protein backbones of 31 and 34 kD, respectively.

In this study we demonstrate that two of the three Thy-1$^+$ DEC-derived cell lines investigated express a T3-associated disulfide-linked heterodimer composed of a 34-kD γ chain and a 46-kD partner (termed the δ chain). The 34-kD γ chain has a protein

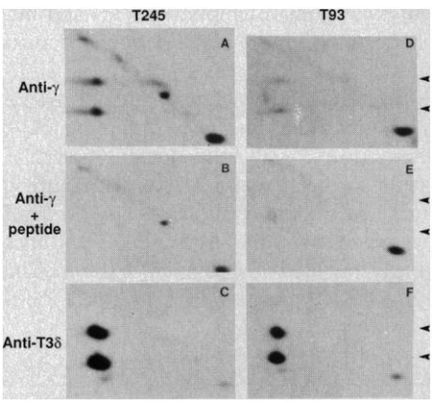

Fig. 2. Analysis of TCR γ-containing receptors on T3$^+$ cell lines T245 (A to C) and T93 (D to F) on two-dimensional nonreducing and reducing SDS-PAGE gels. Cells were surface-iodinated and lysed in either NP-40 (0.5%) or digitonin lysis buffer. Precipitations with the antiserum to the γ chain [specific for the carboxyl termini of Cγ1, Cγ2, and Cγ3 (*5*)] were carried out with the NP-40 lysate (A, B, D, and E), precipitations with anti-T3δ (C and F) were carried out with the digitonin lysate. The positions of 34- and 46-kD molecules, as calculated from the mobility of molecular weight markers, are indicated by arrowheads on the right. (**A**) T245, NP-40 lysate, anti-γ; (**B**) T245, NP-40 lysate, anti-γ in presence of γ peptide; (**C**) T245, D1G lysate, anti-T3δ; (**D**) T93, NP-40 lysate, anti-γ; (**E**) T93, NP-40 lysate, anti-γ in presence of γ peptide; and (**F**) T93, D1G lysate, anti-T3δ. Surface iodination and immunoprecipitation were carried out as described in the legend to Fig. 1. Immunoprecipitates in digitonin lysis-wash buffer were washed with digitonin buffer, whereas immunoprecipitates in NP-40 lysis-wash buffer were washed in NP-40 buffer. The samples were first run on cylindrical SDS-PAGE gels under nonreducing conditions (horizontal, from left to right). These gels were, after equilibration with 2-mercaptoethanol, applied to SDS-PAGE slab gels and run in the second dimension under reducing conditions (vertical, from top to bottom).

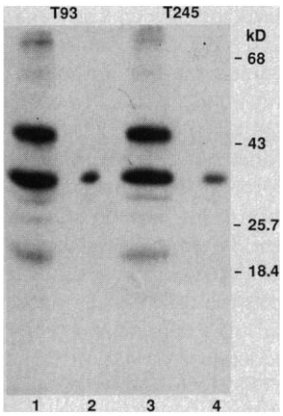

Fig. 3. Only the 34-kD molecule is reactive with anti-γ. Anti-T3ε [a monoclonal antibody reactive with the T3ε chain (*6*)] precipitates with T93 and T245 digitonin cell lysates were reduced, alkylated, and divided into two samples. With one of these samples an anti-γ precipitation was carried out. For reduction alkylation, anti-T3ε precipitates were suspended in 100 μl of NP-40 wash buffer containing 2 m*M* dithiothreitol and 1% SDS and were heated to 68°C for 5 minutes. These samples were then adjusted to 20 m*M* iodoacetamide. All samples were analyzed on SDS-PAGE under reducing conditions. (Lane 1) T93, anti-T3ε precipitation; (lane 2) T93, anti-T3ε, precipitation, followed by reduction, alkylation, and anti-γ precipitation; (lane 3) T245, anti-T3ε precipitation; and (lane 4) T245, anti-T3ε precipitation followed by reduction, alkylation, and anti-γ precipitation.

backbone of 31 kD and probably contains only one *N*-linked glycosyl side chain. The 46-kD molecule has a protein backbone of 34 kD and contains two or more *N*-linked glycosyl side chains. Lew *et al.* (*4*) described a similar T3-associated TCR with a γ chain on a subset of murine thymocytes. Such a TCR complex has also been detected on subsets of human lymphocytes (*7*) and human thymocytes (*8*) and on cloned human natural killer (NK) cells (*9*). Both our studies and those of Lew *et al.* (*4*) demonstrated that, among the murine γ-containing TCR heterodimers, only the lower molecular weight protein was reactive with anti-γ. Furthermore, the γ and δ chains on both thymocytes (*4*) and Thy-1$^+$ DEC cell lines are glycosylated in a similar fashion. The receptors on these two different T cell populations, therefore, appear to have very similar characteristics. It is not yet clear, however, whether the Thy-1$^+$ DEC cells originate from the thymus or extrathymically.

In contrast to T93 and T245, which expressed the γδ receptor, T195 expressed a T3-associated receptor resembling that detected on αβ-expressing cells (*10*) and that could not be precipitated with anti-γ. However, preliminary results indicate that T195 expresses only truncated α-chain and no β-chain messenger RNA. The nature of this receptor is therefore unclear.

So far only six murine TCR γ variable regions have been described (*11*), an indication that the number of different TCRs with a γ chain is limited. In our initial studies on Thy-1$^+$ DEC-derived cell lines, the γ chain appeared to be expressed as a homodimer (*2*). Expression of similar TCRs, in which the γ chain is expressed in the apparent absence of a δ chain, has also been described for human NK cells (*9*) and a human leukemic T cell line (*12*). The apparent ability of the TCR γ chains to form homodimers, in addition to heterodimers, may be a mechanism for increasing the diversity of such TCRs.

One of the most striking observations relating to γ gene expression is that these genes are the first TCR genes to be transcribed and translated during thymic ontogeny (*5, 13*). Later in ontogeny, in the adult thymus and in the periphery, only a very minor subpopulation of lymphocytes appears to express the γ chain (*4, 5, 7–9*). Our results [(*2*) and this study] demonstrate that Thy-1$^+$ DEC cell lines can express the TCR γ chain (certainly four and probably five of the six cell lines examined express γ) and suggest that Thy-1$^+$ DEC cells may express such receptors in vivo and thereby represent one of the major extrathymic T cell populations with this phenotype. The presence of comparatively large numbers of TCR γ chain–expressing cells in the epidermis, a tissue that also contains potent Ia$^+$ antigen–presenting cells and is capable of secreting a diverse array of cytokines, may be important for furthering our understanding of the function of these receptors and the cell populations expressing them.

Note added in proof: The T195 cell line expresses full-length TCR Cγ4 messenger RNA, suggesting that the receptor on this cell line may contain a Cγ4 peptide chain.

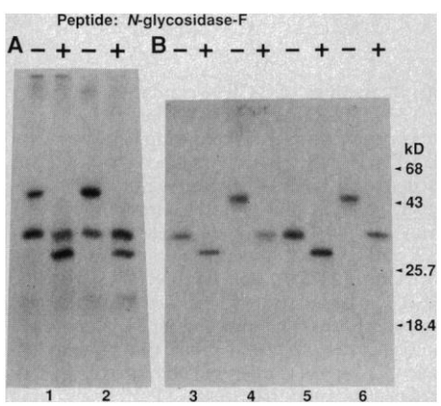

Fig. 4. Analysis of glycosylation of the 34- and 46-kD TCR chains of T93 and T245. (**A**) Anti-T3δ precipitations from digitonin lysates of (lane 1) T93 and (lane 2) T245 were mock-digested (−) or treated with peptide:*N*-glycosidase-F (Genzyme, Boston, Massachusetts) (+) and analyzed on SDS-PAGE under reducing conditions. (**B**) The 34- and 46-kD TCR molecules of T93 and T245 were isolated by cutting out gel slices containing these molecules, followed by electroelution and ethanol precipitation of the proteins. These molecules were subsequently mock-digested or treated with peptide:*N*-glycosidase-F as indicated and analyzed on SDS-PAGE under reducing conditions. (Lane 3) T93, 34-kD molecule; (lane 4) T93, 46-kD molecule; (lane 5) T245, 34-kD molecule; (lane 6) T245, 46-kD molecule. For the peptide:*N*-glycosidase treatment, immunoprecipitates were washed three times with 1% digitonin wash buffer and then once with 0.1*M* sodium phosphate buffer (*p*H 8.6). The PAA beads were suspended in 50 μl of 0.1*M* sodium phosphate buffer plus 0.5% SDS (*p*H 8.6) and boiled for 3 minutes. After the beads were centrifuged, the supernatant was removed and split into two samples. These samples were adjusted to 1% NP-40; then 6 μl of a mixture containing 50% glycerol and 2.5 m*M* EDTA was added to one of these samples (mock digestion), and 6 μl of 50% glycerol and 2.5 m*M* EDTA containing 1.5 U of peptide:*N*-glycosidase was added to the other sample. After 16 hours at 37°C, an equal volume of double-strength sample buffer (*15*) was added to the samples before analysis on SDS-PAGE gels. Isolation, enzyme treatment, and analysis of individual chains were carried out as follows. Immunoprecipitates were analyzed on SDS-PAGE under reducing conditions. The position of the band of interest was determined by autoradiography and excised from the gel, then electroeluted in 5 m*M* tris and 0.1% SDS (*p*H 7.8). The electroeluted protein was precipitated with ethanol (16 hours at −30°C with 9:1 ratio of ethanol to sample) in the presence of carrier protein (cytochrome c) and sodium acetate. The precipitated protein was recovered by centrifugation (30 minutes at 10,000 rev/min), dried, and suspended in 0.1*M* sodium phosphate buffer and 0.5% SDS (*p*H 8.6) and digested with peptide:*N*-glycosidase-F as described above.

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Expression of the art/trs Protein of HIV and Study of Its Role in Viral Envelope Synthesis

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The art/trs transactivator protein of human immunodeficiency virus (HIV) was expressed in mammalian cells as a 19-kilodalton protein that was immunoreactive with sera from HIV-infected patients. Separate plasmids encoding the art/trs protein, the tat protein, or the envelope glycoprotein gp120 were used to demonstrate that both art/trs and tat are absolutely required for the synthesis of gp120 from its cognate messenger RNA. In addition, both the tat and art/trs proteins influence the level of envelope RNA. The results suggest that art/trs and tat may be ideal targets for potential anti-HIV agents in AIDS therapy.

HUMAN IMMUNODEFICIENCY VIRUS (HIV), the cause of the acquired immune deficiency syndrome (AIDS), differs from most known retroviruses in the complexity of its genetic organization. In addition to the long terminal repeats (LTRs) and the *gag*, *pol*, and *env* genes, HIV has several genes that contribute to a system of genetic regulation far more complex than in most retroviruses. Among these are two genes, *tat* and *art/trs*, that are both required for viral replication in vitro (*1–3*). In addition, both genes code for "transactivators" that stimulate expression of other HIV genes. We will refer to the genes as *tat* and *art/trs* and the gene products as tat and art/trs. The *tat* gene product has been expressed as a 14-kD protein in both bacterial (*4, 5*) and mammalian (*6–8*) systems, but the *art/trs* gene product has not previously been identified. One important viral gene regulated by *tat* and *art/trs* is the *env* gene, which encodes the envelope glycoproteins gp120 and gp41. As a component of the viral envelope, gp120 is crucial to the interaction of the virus with its cellular receptor (*9, 10*) and may also contribute to the cytopathogenicity of HIV through its involvement in syncytium formation (*11*). It has been reported that the degree of cytopathic effect of the virus directly correlates with the amount of viral envelope protein synthesized by an infected cell (*8*). The mechanisms by which *tat* and *art/trs* regulate *env* gene expression are not clearly understood. Both tat and art/trs appear to act, at least in part, post-transcriptionally. In *tat* or *art/trs* defective proviral mutants there appears to be substantial viral RNA produced after proviral transfection, although viral protein levels are greatly reduced (*3, 8, 12*), suggesting the involvement of translational control. The *tat* gene product appears to have a bimodal function. In several systems, *tat*-mediated increase of test proteins was greater than can be accounted for by increases in the corresponding messenger RNA (mRNA). This has been interpreted to mean that translational efficiency is an important component of *tat*-mediated transactivation [(*6, 13*); for a review see (*14*)]. The relative contributions of mRNA levels versus translational efficiency in transactivation may depend on the experimental system used. For example, cell type–specific factors may play an important role in determining the final level of transactivation (*6*).

It has been difficult to assign precise roles to the tat and art/trs proteins in part because the two genes overlap (in different reading frames) with each other and with the envelope gene (see Fig. 1). Studies of *tat* or *art/trs* proviral deletion mutants have been difficult to interpret because of the possibility of more than one functional unit being altered simultaneously and because other viral genes may influence the results. Studies that examine the effects of *tat* or *art/trs* on the HIV LTR-directed synthesis of heterologous gene products may not be valid models if translational regulation that relies on mRNA structure is involved. In the study reported here, we investigated the regulation of gp120 synthesis by tat and art/trs using the simplified approach of introducing the separately cloned *env*, *tat*, and *art/trs* genes fused to the HIV LTR into mammalian cells in culture and assessing the contribution of these genes individually and in combination.

Previous studies with *art/trs* have been hampered by the inability to detect the gene product in infected cells or in cells transfected with proviruses. To achieve high levels of expression of *tat* and art/trs, we cloned the *art/trs* and *tat* coding regions derived separately from the viral complementary DNA (cDNA) clone pCV-1 (*15*) (Fig. 1B) into the plasmid pCEV, which utilizes the HIV LTR as a promoter and RNA processing signals from SV40 to express the inserted DNA as a functional mRNA (Fig. 1C). The *art/trs* construction (pART) is capable of expressing only art/trs and not tat because the ATG start codon and much of the first coding exon for *tat* have been deleted. The *tat* construction (pTAT) is capable of expressing only tat and not art/trs because it does not contain sequences following the Bam HI site in the coding region previously shown to be required for *art/trs* function (*3*). Cos-7 cells were transfected with pART or pTAT and protein extracts were made 48 hours later and analyzed by Western blot by using pooled serum samples from HIV-infected patients. Figure 2A shows that a protein of approximately 19 kD is apparent only in pART-transfected cells and not in mock transfected cells or in cells transfected with pTAT. The 19-kD protein does not react with normal human serum. We conclude that this protein is the product of the *art/trs* gene of HIV. The calculated molecular size of art/trs based on the deduced amino acid sequence is approximately 13.6 kD. The higher apparent molecular size that we observe may be due to the high proline content of the protein, which is thought to decrease the mobility of certain proteins in SDS-acrylamide gels (*4, 16*). These results show that the *art/trs* gene can be expressed at high levels as a 19-kD protein that is immunoreactive with sera from HIV seropositive patients.

Because we were unable to detect *tat* protein by Western blot, expression of *tat* was monitored by measuring its ability to transactivate the HIV LTR fused to the chloramphenicol acetyltransferase (CAT) gene. The plasmid pLTR-CAT (Fig. 1C) was cotransfected with pTAT or pART and the level of CAT activity was measured in the protein extract. Increased CAT activity over control levels is indicative of *tat* expression (*17–19*). Figure 2B shows that transactivation of the HIV LTR occurs only when pTAT is transfected, indicating that pTAT expresses the tat protein. Figure 2 also confirms that only the expected viral gene products are made from the *tat* and *art/trs* plasmids. When pART is cotransfected with

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