suggest that the disease may have originated in the New World and entered the Old World after 1492 but before 1785. We further propose that rheumatoid arthritis may derive from pathogens or allergens originally native to the New World. This interpretation is more parsimonious than the alternative of a parallel, independent evolution of a now-extinct unknown New World polyarthritis which also produced a constellation of osseous attributes distinct to rheumatoid arthritis.

Items present in both the Late Archaic lifeway and the Colonial period transatlantic trade are potential agents of transportation of this disease. Our consideration of the Late Archaic lifeway and of the temporal and geographic patterns of early transatlantic trade (*26*) implicates tobacco, deer, people, rodents, and dogs as most likely to be associated with a vector, pathogen, or allergen responsible for rheumatoid arthritis.

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Antiproliferative Activity of a Hybrid Protein Between Interferon-γ and Tumor Necrosis Factor–β

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A hybrid protein between interferon-γ and tumor necrosis factor–β was made by ligating the respective genes and expressing the fused genes under the control of the *trp* promoter in *Escherichia coli*. The antiproliferative activity of the hybrid protein in vitro was greatly increased compared with either interferon-γ or tumor necrosis factor–β alone, and both antiviral activity and cytotoxic effect were retained in the hybrid protein. The hybrid protein may have potential clinical application.

COMBINED TREATMENT OF TUMOR cells with tumor necrosis factors (TNFs) and interferons (IFNs) has resulted in a synergistic anticellular effect in many cases (*1–5*). In some tumor cell lines, growth inhibition only occurs after treatment with a combination of both lymphokines (*3, 5*). Synergism between IFNs and TNFs has been observed even in a TNF-resistant tumor cell variant (*6*). On the basis of these observations we constructed two plasmids in which the IFN-γ and TNF-β (lymphotoxin) genes were juxtaposed so that they would form a possible fusion protein under the control of the *Escherichia coli trp* promoter. We report here that one of these constructs expresses a hybrid protein with both IFN-γ and TNF-β activity, and this protein has a greater antiproliferative effect on the tumor cell line ME-180 in vitro than either lymphokine alone.

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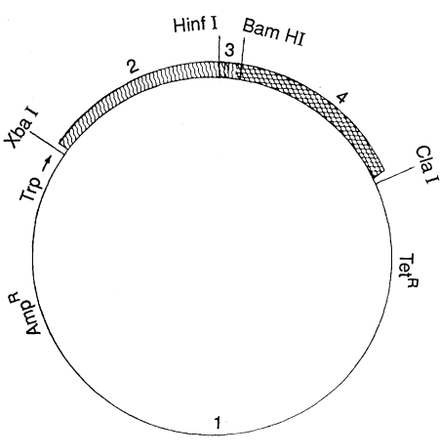

Fig. 1. Plasmid structure for IFN-γ–TNF-β fusion protein expression. Region 1, 4660-bp Xba I–Cla I vector fragment from pγ143 (*18*); region 2, 406-bp Xba I–Hinf I fragment coding for IFN-γ from pγ143 (*18*); region 3, synthetic DNA to link and fuse coding region; region 4, 597-bp Bam HI–Cla I fragment coding for TNF-β from pLT *trp*-1 (*17*). The two plasmids differ in the synthetic DNA fragment region used to link the two coding regions. Plasmid pγTNF-β1 contains the synthetic sequence AGT CAG ATG CAC TCA ACT CTG AAG CCA GCA GCA CAC CTG ATC GGG, and therefore codes for the first 134 residues for IFN-γ (*7*) and the last 148 residues of TNF-β (*5*). Plasmid pγTNF-β2 contains additional 27 nucleotides (CTG TTT CGA GGT CGA AGA GCA TCC CAG) between base 9 and base 10 of the above sequence. Consequently, pγTNF-β2 codes for the entire length (143 residues) of human IFN-γ fused to the 148 residues of TNF-β.

The 13 carboxyl-terminal residues of IFN-γ are unnecessary for biological activity (*7*). Similarly, the 23 amino-terminal residues of TNF-β are not required for bioactivity (*5*). Consequently, a hybrid gene was designed to encode a fusion protein of IFN-γ at the amino-terminal end and TNF-β at the carboxyl-terminal end by removing these sequences. A second plasmid containing an intact IFN-γ gene fused to the truncated TNF-β gene was also made. Plasmids containing the intact IFN-γ gene and the truncated TNF-β gene were used as controls. The structure of the recombinant plasmids is shown in Fig. 1.

To determine that the fusion proteins retained both IFN-γ and TNF-β activities, we showed that the *E. coli* extracts contained material that could protect WISH and Hep-2 cells when challenged with encephalomyocarditis (EMC) virus and vesicular stomatitis virus (VSV) and that also had a cytotoxic effect on mouse L929 cells (Table 1). There was no significant difference in the antiviral activity of extracts of *E. coli* strains harboring plasmids pγTNF-β1 or pγTNF-β2 and that of *E. coli* containing the IFN-γ gene. All of the antiviral activity produced by the hybrid constructs could be neutralized by antibody to IFN-γ but not by antibody to IFN-α or IFN-β (*8*). When tested for cytotoxicity on mouse L cells, both extracts from pγTNF-β1 and pγTNF-β2 were cytotoxic to the same extent. We would thus conclude that both activities were present in the cell lysates.

To determine whether these activities were contained in the same molecule or were processed by *E. coli* proteolytic enzymes to two independent activities, we purified the extracts on IFN-γ monoclonal antibody affinity columns. The column was washed with phosphate-buffered saline, and samples were eluted at high salt (1*M* NaCl and 50% ethylene glycol) as described (*9*). IFN-γ and TNF-β activities were coeluted from the extract containing pγTNF-β1. However, some processing had apparently taken place in pγTNF-β2, since much of the TNF-β activity was separated from that of IFN-γ (*10*).

The above results were confirmed by immunoblot analysis with monoclonal antibody to IFN-γ and polyclonal antibody to TNF-β (Fig. 2). Only one band, a 30-kD protein, is obvious in extracts of pγTNF-β1; this protein reacts with both antibody to IFN-γ and antibody to TNF-β. However, in the preparations from pγTNF-β2, there are a number of bands that are barely visible:

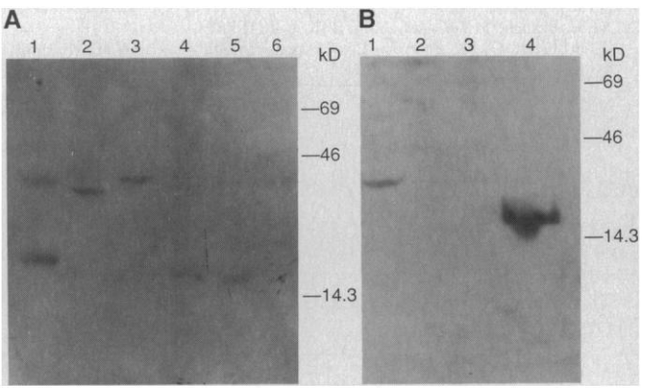

Fig. 2. Immunoblot analysis of bacterial extracts. (**A**) The first antibody was mouse monoclonal antibody to human IFN-γ and the second antibody was ^{125}I-labeled sheep F(ab')$_2$ fragment of antibody to mouse immunoglobulin (Ig). Lane 1, purified IFN-γ (Genentech); lane 2, pγTNF-β1; lane 3, pγ143; lane 4, pγTNF-β2; lane 5, pTNF-β; and lane 6, pBR322. (**B**) First antibody was rabbit antibody to human TNF-β and second antibody was ^{125}I-labeled donkey F(ab')$_2$ fragment of antibody to rabbit Ig. Lane 1, pγTNF-β1; lane 2, pγ143; lane 3, pγTNF-β2; and lane 4, pTNF-β. Molecular marker proteins: lysozyme, 14.3 kD; ovalbumin, 46 kD; and bovine serum albumin, 69 kD. Protein samples were first run on a discontinuous SDS-polyacrylamide gel, the separating gel containing 12.5% acrylamide and the stacking gel, 3% acrylamide. After electrophoresis, the gel was soaked in transfer buffer (25 m*M* tris, 192 m*M* glycine, and 20% methanol) for 30 min at room temperature. The nitrocellulose paper was also equilibrated in transfer buffer. The proteins in the gel were blotted onto nitrocellulose paper in an electrical transfer apparatus overnight at 4°C (*19*). The nitrocellulose paper was rinsed with water and air-dried, and the lane containing molecular marker proteins was cut out and stained with India ink (1 μl/ml of phosphate-buffered saline (PBS)–Tween 20). The other part of the paper was blocked for 4 hours at room temperature on a shaker with PBS–Tween 20, incubated with first antibody in PBS–Tween 20 at room temperature 2 to 4 hours and then washed three times with PBS–Tween 20. The second antibody incubation and subsequent washing was similar to the first antibody. The paper was air-dried and exposed on x-ray film at −70°C.

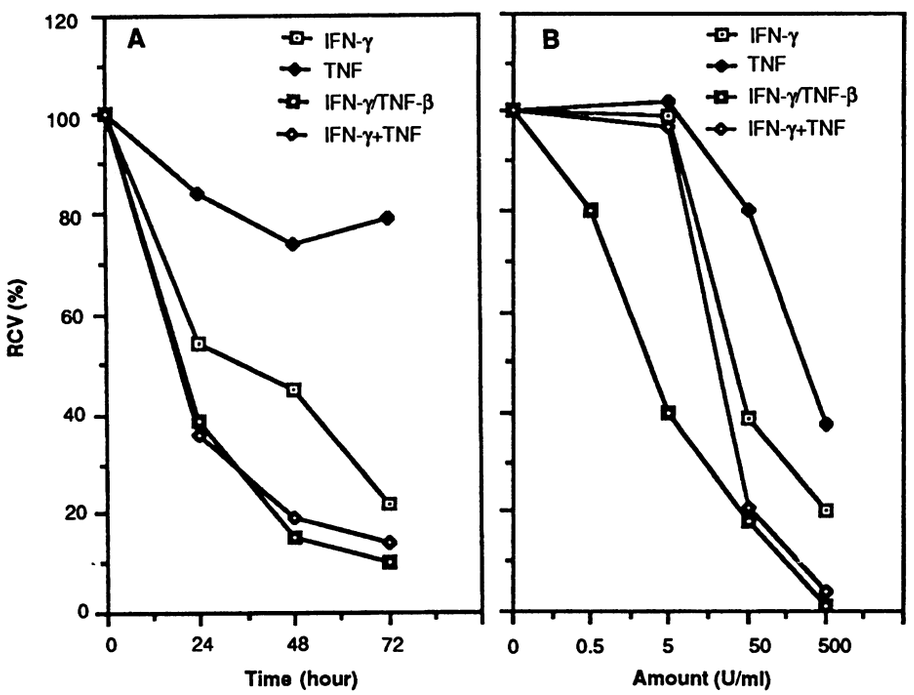

Fig. 3. The enhanced antitumor activity of hybrid IFN-γ–TNF-β purified protein from *E. coli* lysates. (**A**) ME-180 cells (2×10^4 cells per well) were incubated in 96-well plates with purified IFN-γ or TNF at 100 U/ml, and IFN-γ + TNF (100 U of each per milliliter), as well as hybrid IFN-γ–TNF-β (100 U/ml in terms of antiviral activity) for 24, 48, and 72 hours at 37°C. [^{3}H]Thymidine was added (1 μCi per well) for the last 2 hours. Cells were harvested, and the amount of tritium incorporated into DNA was measured. Relative cell viability (percent) represents the ratio (in counts per minute) of treated versus nontreated ME-180 cells. Each point represents the mean of eight replicates and the SD was less than 10% of the mean. (**B**) ME-180 cells were incubated with different amounts of IFN-γ, TNF, and hybrid IFN-γ–TNF-β as shown for 72 hours. Relative cell viability was determined as above. Four replicates were used for each treatment group and the SD was less than 10% of the mean.

Table 1. Biological activities of hybrid proteins in crude *E. coli* cell extracts. Cultures of transformed *E. coli* HB101 and their cell extracts were made as described (*15*). Antiviral activity was assayed on human WISH cells challenged with EMC virus or VSV (*16*). All IFN titers were calibrated against the NIH standard IFN samples. TNF activity was measured on L929 cells without pretreatment with actinomycin D or mitomycin (*17*). Both IFN and TNF assay have been performed at least five times. The data shown are the results of representative experiments.

Clone	IFN activity (log_{10} IU/liter)	TNF activity (log_{10} U/liter)
pγTNF-β1	7.11	4.48
pγ143	7.41	0
pγTNF-β2	6.51	4.48
pTNF-β	0	6.28
pBR322	0	0

these bands correspond to molecular sizes of 15, 30, and 35 kD. These results together with the purification data indicate that pγTNF-β1 expresses a true fusion protein, whereas the fusion protein in pγTNF-β2 has been processed. Purified recombinant IFN-γ always gives two bands in SDS-polyacrylamide gels, one of 17 kD and another of 35 kD. This probably reflects the formation of covalent dimers. TNF-β migrates with a molecular size of 15.5 kD.

We further tested the anticellular activity of the fusion protein IFN-γ–TNF-β1 on ME-180, a human cervical cancer cell line. This cell line is sensitive to both IFN-γ and TNF and hypersensitive to a combination of these lymphokines. The anticellular effect of the hybrid IFN-γ–TNF-β1 protein is greater than that of either lymphokine by itself or the combined effect of both lymphokines at lower levels (Fig. 3).

In both pγTNF-β1 and pγTNF-β2, the first 23 amino acids of TNF were removed. However, in pγTNF-β2 the whole sequence of IFN-γ was retained, whereas in pγTNF-β1 the last nine amino acids were deleted. Natural human IFN-γ is processed at the carboxyl terminus (*11*). Six different termini have been identified and all have at least nine residues deleted. Thus this region of the IFN may form a sensitive site for proteolytic cleavage in the bacterial cell. This region contains two tandem arginine residues, and it is possible that this is a target for *E. coli* proteolytic enzymes (*12*).

Combined treatment with IFNs and TNFs results in a synergistic anticellular effect in many tumor cell lines (*1–5*). In ME-180 cells, this synergism may be related to upregulation of the number of receptors for TNFs by IFN-γ (*6, 13*). Epidermal growth factor and recombinant human transforming growth factor–β interfered specifically with the antiproliferation effects of TNFs but not with those of IFN-γ on ME-180 cells; neither growth factor had a substantial protective effect on the synergistic cytotoxicity of TNF and IFN-γ, implying that IFN-γ and TNFs inhibit tumor cell growth by distinct mechanism (*14*). We observed that very low levels of the hybrid IFN-γ–TNF-β protein caused an enhanced cytotoxic effect on ME-180 cells. This intramolecular synergism between the IFN-γ domain and the TNF-β1 domain within the hybrid protein may be different from the synergism between these two separate lymphokines, since the antiproliferative activity of IFN-γ–TNF-β was even higher than that of a combination of IFN-γ and TNF-β at lower levels (Fig. 3). At this time we do not know the exact mechanism whereby the hybrid molecule induces this enhanced antiproliferative activity, although this is possibly due to the formation of an unusual complex between the hybrid molecule and the receptors for TNF and IFN-γ on the cell surface. However this enhanced activity suggests a potential for clinical application of the hybrid protein.

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Hormone-Sensitive Lipase: Sequence, Expression, and Chromosomal Localization to 19 cent-q13.3

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Hormone-sensitive lipase, a key enzyme in fatty acid mobilization, overall energy homeostasis, and possibly steroidogenesis, is acutely controlled through reversible phosphorylation by catecholamines and insulin. The 757–amino acid sequence predicted from a cloned rat adipocyte complementary DNA showed no homology with any other known lipase or protein. The activity-controlling phosphorylation site was localized to Ser^{563} in a markedly hydrophilic domain, and a lipid-binding consensus site was tentatively identified. One or several messenger RNA species (3.3, 3.5, or 3.9 kilobases) were expressed in adipose and steroidogenic tissues and heart and skeletal muscle. The human hormone-sensitive lipase gene mapped to chromosome 19 cent-q13.3.

FREE FATTY ACIDS (FFA) DERIVED from adipose tissue triglycerides are the most important fuel in mammals and provide more than half of the caloric needs when dietary energy substrates are lacking. Hormone-sensitive lipase (HSL) has a vital role in the mobilization of FFA from adipose tissue by controlling the rate of lipolysis of the stored triglycerides. Like glycogen phosphorylase, the corresponding enzyme in carbohydrate metabolism, HSL is under acute neuronal and hormonal control.

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