Inhibition of Development of Exoerythrocytic Forms of Malaria Parasites by γ-Interferon

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A specific DNA probe was used to study the effect of recombinant rat, mouse, and human γ-interferon (γ-IFN) on the course of sporozoite-induced malaria infections. In mice and rats infected with sporozoites of *Plasmodium berghei*, mouse and rat γ-IFN's strongly inhibited the development of the exoerythrocytic forms in the liver cells of the hosts, but not the development of the erythrocytic stages. The degree of inhibition of the exoerythrocytic forms was proportional to the dose of γ-IFN administered, but was independent of the number of sporozoites used for challenge. A 30 percent reduction in the development of exoerythrocytic forms in rat liver was achieved when 150 units (about 15 nanograms of protein) of rat γ-IFN were injected a few hours before sporozoite challenge; the reduction was 90 percent or more with higher doses of γ-IFN. The effect was less pronounced if the γ-IFN was administered 18 hours before or a few hours after challenge. Human γ-IFN also diminished the parasitemia in chimpanzees infected with sporozoites of the human malaria parasite *Plasmodium vivax*. The target of γ-IFN activity may be the infected hepatocytes themselves, as shown by in vitro experiments in which small doses of the human lymphokine inhibited the development of exoerythrocytic forms of *Plasmodium berghei* in a human hepatoma cell line. These results suggest that immunologically induced interferon may be involved in controlling malaria infection under natural conditions.

MALARIA INFECTION STARTS when mature sporozoites present in the salivary glands of *Anopheles* mosquitoes are inoculated into the blood of vertebrate hosts. After a few minutes in circulation, the sporozoites invade hepatocytes. By schizogony, each sporozoite generates a progeny of several thousand merozoites inside a parasitophorous vacuole. Upon rupture of the infected hepatocytes, the released merozoites invade erythrocytes and start a new schizogonic cycle that is associated with the clinical symptoms of malaria.

Because infected hepatocytes do not display parasite-derived or other neoantigens on their membranes, the exoerythrocytic forms (EEF) are protected from direct attack by antibodies. However, administration of inducers of α- and β-interferons, such as Newcastle disease viruses and double-stranded RNA, reduce the severity of sporozoite-induced *Plasmodium berghei* infections in mice, presumably by affecting the EEF (*1*). We have now studied the direct effect of γ (or immune) -IFN on sporozoite-induced malaria. We reasoned that this type of lymphokine, produced by activated T lymphocytes upon their encounter with antigen (*2*), would be more likely to play a role in the naturally acquired resistance to malaria infection than α- and β-interferons.

In the initial experiments (Fig. 1) we injected five groups of five A/J mice (Jackson Laboratory) intravenously with doses of 200 to 2×10^5 units of mouse recombinant γ-IFN (*3*) 5 hours before challenging them with 5×10^3 *P. berghei* sporozoites. Blood samples were assayed for the presence of parasite DNA by using a 2.3-kb, ^{32}P-labeled repetitive DNA probe (p263-1) specific for *P. berghei*. The lower limit of sensitivity of the assay was 100 pg of parasite DNA, equivalent to approximately 10^3 haploid nuclei (*4*). This permitted detection of parasites in the blood of control animals only 2 days after challenge, when the merozoites had just emerged from the EEF and entered the red cells. The prepatent period of the infection was increased in all mice that received γ-IFN. Although all treated animals had parasites in the blood on the sixth day, the levels of parasite DNA were 1 to 10 percent of the controls, even when the injected dose of γ-IFN was only 200 U. In other experiments we found that the γ-IFN effects were neutralized by specific antibodies (*5*).

In contrast, the injection of 10^5 U of γ-IFN at 72 hours after sporozoite challenge (a time when the parasites had left the liver and already entered the red blood cells) had no statistically significant effect (tested by two-tailed *t* tests) on the time course of parasitemia when compared to untreated controls. For example, at 120 and 144 hours after challenge, the micrograms of *P. berghei* DNA per gram of blood (mean ± SD) in IFN-treated and untreated groups were, respectively, 2.63 ± 3.09 versus 5.12 ± 4.24 and 14.75 ± 13.49 versus 19.27 ± 9.2. These findings suggested that the effect of γ-IFN was exclusively on the EEF. To demonstrate this point, we used as experimental animals juvenile Norway Brown rats because a larger proportion of *P. berghei* sporozoites penetrate hepatocytes and develop into EEF in these animals (*4, 6*). This fact, together with the use of the p263-1 DNA probe, permitted direct measurement of the effects of IFN on the EEF, even though the amount of parasite DNA was only a minor fraction of the total liver DNA (*4*). In these studies, we injected recombinant rat γ-IFN (*7*) into the animals at different times after challenge with 10^5 sporozoites (time 0). The amount of parasite DNA was measured in the livers at 44 hours after challenge, when EEF development was maximal (*4*).

The γ-IFN treatment strongly inhibited the EEF development, particularly when administered at −5 hours (Fig. 2). In two separate experiments, 150 U of γ-IFN (10 to 20 ng of protein) inhibited approximately 30 percent of EEF development, measured at 44 hours after challenge. Close to 90 percent inhibition was reached with 6×10^4 U of γ-IFN. The effect was reversed by mixing the lymphokine with specific antibodies to rat γ-IFN (*8*).

The inhibition was less marked if rat γ-IFN was injected 18 hours before or after sporozoite challenge. For example, the percent inhibition (mean ± SD) achieved with 5×10^5 U of γ-IFN given at +5 hours (72 ± 10 percent) was not significantly different (two-tailed *t* test) from that obtained with 1.5×10^4 U at −5 hours (73 ± 10 percent). However, significant inhibition was observed even when the γ-IFN was injected 20 hours after challenge, when the parasite had undergone several cycles of nuclear divisions in the hepatocyte (*4*). Although small doses of γ-IFN were required for 90 percent or more inhibition of EEF development, the parasites were eliminated only when three doses of 5×10^5 U were given at −18, −5, and +24 hours after challenge (Fig. 2).

Next, we asked whether the potency of the inhibitory effect of γ-IFN was dependent on the dose of sporozoites. We injected groups of six rats with 1.5×10^4 U of γ-IFN, a dose previously shown to inhibit 70 percent of EEF development in animals infected with 10^5 sporozoites. Five hours after injection of γ-IFN, these rats were challenged with 40,000, 120,000, and 400,000 *P. berghei* sporozoites. The amounts of parasite DNA present in the

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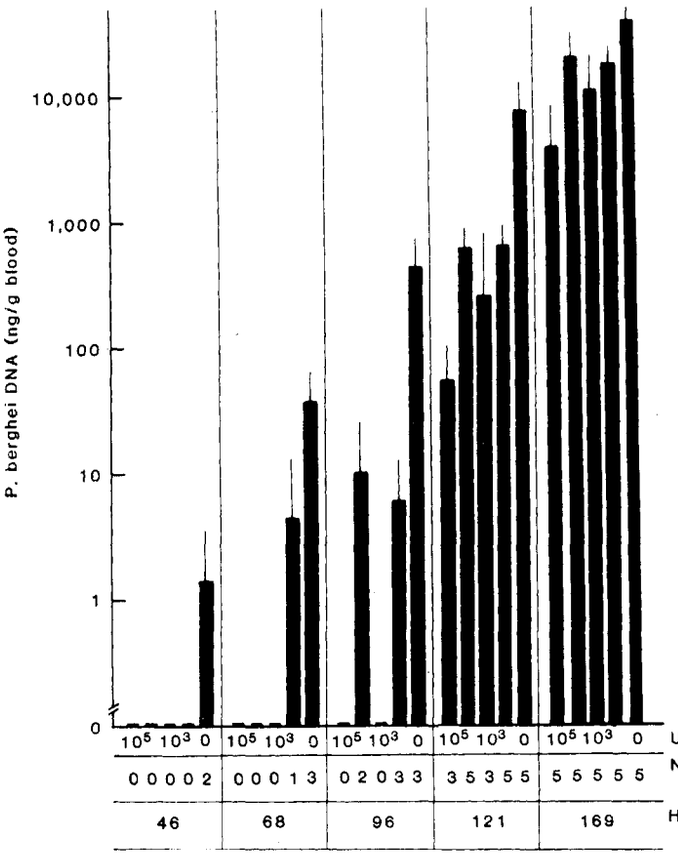

Fig. 1. Parasitemia in mice treated with recombinant murine γ-IFN and infected with *P. berghei* sporozoites. Female A/J mice (2 to 3 months old) were injected with dilutions of γ-IFN, or with phosphate-buffered saline (PBS), *p*H 7.2, 5 hours before the intravenous injection of 5×10^3 sporozoites of *P. berghei* obtained by dissection of the salivary glands of *Anopheles stephensi* mosquitoes (*22*). The amount of parasite DNA present in the blood of these animals was measured as described (*23*). Blood smears were also prepared and examined for the presence of parasitized red blood cells.

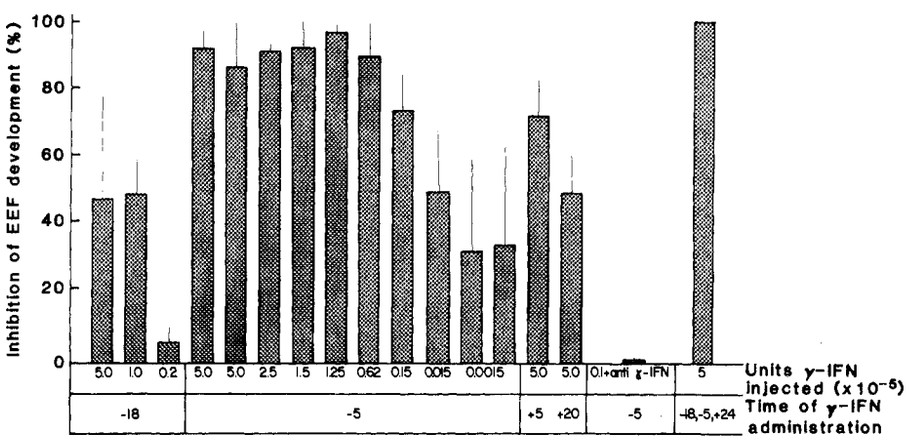

Fig. 2. Inhibition of the development of *P. berghei* EEF by recombinant rat γ-IFN. Groups of four to six female Norway Brown rats (Charles River), 2 to 4 months old, were injected intravenously with different doses of rat γ-IFN, at different times before or after the intravenous injection of 10^5 *P. berghei* sporozoites. The γ-IFN was diluted in 1% heat-inactivated (56°C, 30 minutes) normal rat serum. The figure summarizes the results of several experiments; each one is compared to its own positive control, obtained by treating the rats with γ-IFN diluent. Forty-four hours after the rats were challenged with sporozoites, blood was obtained from the axillary vein and artery, and the livers were removed. At this time the development of EEF in the liver is maximal (*4*). The DNA was purified from the liver and probed for the presence of *P. berghei* DNA (*4*). The amount of parasite DNA per liver was calculated with respect to a standard curve, constructed, and used as described in the legend to Fig. 1. Values, expressed as means and standard deviations, represent the percent inhibition of EEF development and were obtained by comparing the amounts of hepatic parasite DNA present in γ-IFN–treated and control groups. The time of γ-IFN injection is expressed in hours relative to the time of sporozoite injection (time 0). All groups except that on the extreme right received single IFN injections. In one experiment (second bar from right) the γ-IFN was diluted in two neutralizing doses of a polyclonal rabbit antibody to rat γ-IFN (*8*) prior to injection into rats. As a control, the γ-IFN was incubated with an equivalent amount of normal rabbit serum.

liver of rats treated with γ-IFN were compared with those present in the respective untreated controls. The percent inhibition (mean ± SD) of EEF development at 44 hours after challenge were, respectively, 72 ± 15, 59 ± 16, and 64 ± 5. No statistically significant differences among the means of the groups were found by one-way analysis of variance.

We then tested the effect of recombinant human γ-IFN (*9*) on EEF of *P. vivax*, [Chesson strain (*10*)] Two chimpanzees (*Pan troglodytes*) received daily doses of 5×10^6 U of γ-IFN for 6 days, starting 5 hours before they were challenged with a relatively large number (10^5) of sporozoites. On day 7, these two chimpanzees and two others that received sporozoites but no γ-IFN were splenectomized to permit the development of the blood stages. Starting at day 8, blood smears were examined for the presence of parasites. As shown in Fig. 3, the prepatent periods in the control animals were 10 and 12 days, and in the experimental animals 12 and 16 days. The parasitemias were much reduced in the γ-IFN–treated animals until day 16, when curative treatment with chloroquine and primaquine was started. In agreement with previous studies, the chimpanzees suffered no ill effects from γ-IFN administration (*11*).

We also studied the effect of recombinant human γ-IFN on the development of *P. berghei* in vitro, using the human hepatoma cell line HEP G2-A16 (*12*). Treatment of the target cells 24 hours before sporozoite invasion with recombinant human γ-IFN at concentrations greater than 1 U/ml inhibited the multiplication of parasites by 97 percent. In cells exposed to 1 U/ml, EEF multiplication was reduced by 83 percent. Significant inhibition was observed even with 0.01 U/ml (Fig. 4).

The inhibitory activity of γ-IFN in vivo could be measured accurately with a specific DNA probe because EEF are found exclusively in the host's liver cells. Maximum (but not total) inhibition of EEF development was achieved with 0.62×10^5 U, but significant effects were observed with doses as low as 150 U (~15 ng of protein). To our knowledge, the remarkable activity against intracellular parasites of this very low dose of γ-IFN in vivo has only one reported precedent: the intraperitoneal injection of less than 100 U of γ-IFN into mice activated the oxidative metabolism of resident macrophages and their ability to inhibit the growth of *Toxoplasma gondii* and *Leishmania donovani* in vitro (*13*).

The activity of γ-IFN was greatest when injected a few hours before challenge with sporozoites. This is in contrast to prior observations (*1*) indicating that IFN induc-

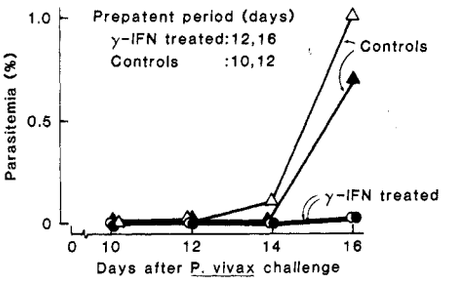

Fig. 3. Inhibition of *P. vivax* infectivity in chimpanzees treated with recombinant human γ-IFN. We used four adult male chimpanzees (*Pan troglodytes*, 55 to 65 kg) from the New York University Laboratory for Experimental Medicine and Surgery in Primates. Two chimpanzees were injected intravenously with 5×10^6 U of γ-IFN (diluted in PBS) at −5 hours, +2 hours, and daily on days 1 through 6; the other two received PBS injections. At time 0, the animals were challenged with 10^5 *P. vivax* sporozoites freshly dissected from the salivary glands of *Anopheles stephensi* mosquitoes. On day 7, the animals were splenectomized. Two blood smears per animal were prepared starting on day 8 and every other day thereafter. A total of 2×10^4 red blood cells was counted in two slides. Results are expressed as percent parasitemia.

ers (for example, Newcastle disease virus) were most effective in protecting mice against malaria if given 20 hours after sporozoite inoculation. The effects with such IFN inducers were probably mediated by α- and β-interferons, which may inhibit EEF by different metabolic pathways. If this view is correct, then the combination of the various interferons (α, β, and γ) could act synergistically to prevent EEF development.

The mechanism by which γ-IFN inhibits EEF development is unknown. γ-IFN activates oxidative metabolism in macrophages as well as increases their antimicrobial activity (*14*). In addition, γ-IFN can destroy parasites by respiratory burst–independent mechanisms, since it prevents the in vitro growth of several microorganisms [*Toxoplasma gondii, Rickettsia prowazekii, Coxiella burnettii*, and *Chlamidia trachomatis* (*15*)] in fibroblasts. Although the possibility that macrophages or natural killer cells recognize the few randomly distributed infected hepatocytes and destroy the parasites cannot be excluded, this seems improbable. Instead, the targets of the lymphokine may be the hepatocytes themselves. We show here that at doses equal to or lower than those required for its antiviral activity, γ-IFN prevented the growth of EEF inside a hepatoma cell line in vitro. That γ-IFN acts on hepatocytes in vivo is also compatible with the observation that its activity is independent of the number of sporozoites used for challenge. We speculate that γ-IFN, either directly or through a lymphokine "cascade," may render the liver cells unsuitable for the development of the malaria parasite.

In the case of *T. gondii*, γ-IFN blocks parasite development by inducing the infected fibroblasts to degrade tryptophan (*16*). Whether γ-IFN also induces tryptophan deficiency or the accumulation of toxic tryptophan metabolites in hepatocytes, thereby arresting EEF development, is not known.

The present findings raise the possibility that interferons play a role in the protective immunity to malaria conferred by vaccination with irradiated sporozoites (*17*) or by repeated exposure to infected mosquitoes in endemic areas. Indeed, the spleen cells of mice immunized with irradiated sporozoites released high levels of γ-IFN when challenged in vitro with antigen (*18*). Interferons have also been detected in the serum of *P. falciparum*–infected patients (*19*). It is therefore conceivable that upon encountering sporozoite antigens the sensitized T lymphocytes release γ-IFN which then acts on the EEF.

A possible application of these findings can be envisaged. Some EEF of *P. vivax* and *P. ovale* remain viable in the human liver for long periods after the elimination of the blood stages (*20*). Primaquine (an 8-amino quinoline) is the only available drug that attacks these liver stages, but its usefulness is offset by innate toxicity and the need for prolonged treatment (*21*). Perhaps recombinant interferons can also destroy the remaining dormant forms of the parasite and prevent clinical relapses.

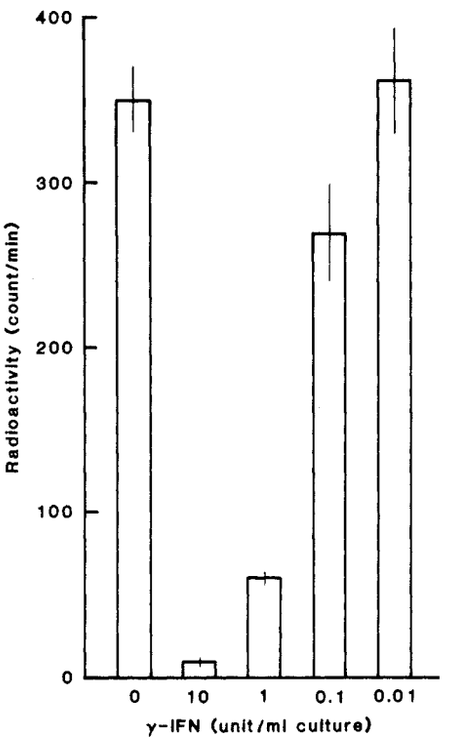

Fig. 4. In vitro inhibition by γ-IFN of *P. berghei* development in the HEP G2.A16 human hepatoma cell line. Cells were grown to confluency in 24-well plates in minimal essential medium (MEM) with 10% fetal calf serum (FCS). Sets of wells in quadruplicate were then exposed to serial dilutions of recombinant human γ-IFN (10 to 0.01 U/ml in 1 ml of MEM + 10% FCS). Control wells received 1 ml of MEM + 10% FCS alone. After 24 hours the volumes in each well were reduced to 200 μl, and 5×10^4 *P. berghei* sporozoites were added. After 3 hours, the wells were washed thoroughly with PBS. One-milliliter volumes of medium containing antibiotics and the appropriate γ-IFN concentration were then added. After 52 hours, corresponding to the peak of parasite proliferation in the in vitro system, the cells were harvested and assayed for the presence of parasite DNA with the p263-1 DNA probe. Similar results were obtained if γ-IFN was added 3 hours after the incubation of sporozoites with the hepatocytes, at a time when the parasites had already invaded the target cells. Background levels were determined by measuring parasite DNA in wells containing hepatoma cells incubated with 5×10^4 heat-inactivated (56°C, 30 minutes) sporozoites; these levels were subtracted from the experimental values. Values are expressed as means and standard deviations.

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5. Three groups of six mice were injected intravenously with 5×10^3 sporozoites. Five hours before, one group (A) had been treated with 2.5×10^5 U of γ-IFN mixed with 2.5 μg of rabbit immunoglobulin G (IgG) antibodies to γ-IFN. At the same time, another group (B) was treated with the same dose of γ-IFN mixed with an equivalent amount of normal rabbit IgG. The third group of mice (C) served as controls for the infectivity of the sporozoites, and they were injected with phosphate-buffered saline (PBS) alone at −5 hours. The blood of all the mice was probed for the presence of parasite DNA 168 hours after challenge. At this time the blood of all mice in groups A and C contained parasites, and the differences in the parasitemias (expressed as the mean and SD of the micrograms of *P. berghei* DNA per milliliter of blood) were not statistically significant between the groups (62.2 ± 48 and 64.5 ± 29, respectively) (tested by two-tailed *t* tests). Only four out of six mice in γ-IFN–treated group B were patent, and the levels of parasitemia (8.0 ± 9.6 μg) were much lower than those in the control group C.
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8. The antibodies to recombinant rat γ-IFN were prepared in rabbits. Partially purified γ-IFN was

subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and about 50 μg of γ-IFN was recovered from gel slices in the 18,000 M_r region. Rabbits were injected with this dose of antigen every 2 weeks for 10 weeks. Six weeks after the last immunization, the animals received booster immunizations consisting of 200 μg of γ-IFN electroeluted from the SDS-PAGE slices. The antiserum contained 10^5 neutralizing units per milliliter.

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23. Blood samples (from the ventral vein and artery of the tail) were centrifuged, the red blood cells were washed once with PBS, resuspended in 1 ml of PBS containing 0.015% (w/v) saponin (Sigma), incubated at 37°C for 5 minutes, and centrifuged. Normal rat liver DNA (50 μg) was added as a carrier to the pellet (parasites plus white cell nuclei). The cell pellet was homogenized with 150 m*M* NaCl, 10 m*M* EDTA, and 1% (w/v) SDS, and sodium perchlorate was added to 0.05*M*. A single extraction with an alcohol-phenol mixture was performed. The DNA in the aqueous phase was ethanol-precipitated and redissolved in water. The DNA in each blood sample was immobilized in 2.5-cm-diameter, 0.45-μm pore size nitrocellulose filters (Millipore) and hybridized with a genomic 2.2-kb repetitive *P. berghei* DNA probe, labeled with ^{32}P by nick translation. Background radioactivity was calculated from filters containing DNA from normal mouse blood and carrier rat DNA. A standard hybridization curve was constructed by probing triplicate tenfold dilutions of blood-stage *P. berghei* DNA. A linear relation between the radioactivity associated with the filters and the amount of parasite DNA immobilized was always obtained in the range of 100 pg to 1 μg of *P. berghei* DNA. The amounts of parasite DNA associated with the experimental filters were calculated by referring their specific radioactivity to the standard curve and expressing them as the means of the nanograms of *P. berghei* DNA detected in five animals.

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Deregulation of c-*myc* by Translocation of the α-Locus of the T-Cell Receptor in T-Cell Leukemias

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Two human T-cell leukemias carrying a t(8;14)(q24;q11) chromosome translocation were studied for rearrangements and expression of the c-*myc* oncogene. For one leukemia, rearrangement was detected in a region immediately distal (3′) to the c-*myc* locus; no rearrangements of c-*myc* were observed in the second case (DeF). However, studies with hybrids between human and mouse leukemic T cells indicated that in the leukemic cells of DeF, the breakpoint in chromosome 14 occurred between genes for the variable (V_α) and the constant (C_α) regions for the α chain of the T-cell receptor. The C_α locus had translocated to a region more than 38 kilobases 3′ to the involved c-*myc* oncogene. Since human c-*myc* transcripts were expressed only in hybrids carrying the 8q+ chromosome but not in hybrids containing the normal chromosome 8, it is concluded that the translocation of the C_α locus 3′ to the c-*myc* oncogene can result in its transcriptional deregulation.

SOME HUMAN T-CELL MALIGNANCIES carry specific chromosome rearrangements, predominantly translocations and inversions, that involve chromosome region 14q11.2 (*1*), the location of the locus for the α-chain of the T-cell receptor (*2*). One of the most common chromosome alterations in acute lymphocytic leukemia of the T-cell type is a t(11;14)(p13;q11) chromosome translocation (*3*). We have shown previously that the chromosome break at band 14q11 in these tumors directly involves the locus for the T-cell receptor between the genes for the variable (V_α) and for the constant (C_α) regions of the α-chain of the T-cell receptor and that the V_α genes are proximal and the C_α gene is distal to the 14q11 chromosome breakpoints (*4*). Thus the orientation of the α-locus of the T-cell receptor relative to the centromere is the opposite of that of the human immunoglobulin heavy-chain locus on chromosome 14 (*5*).

Recently a translocation between chromosome 8 and 14, with breakpoints at 8q24 (the locus of the c-*myc* oncogene) (*6*), and 14q11 (the locus for the α-chain of the T-cell receptor) (*2*), has been described in several T-cell neoplasms (*7–8*). These findings suggest that the locus for the α-chain may be involved in c-*myc* deregulation in some T-cell malignancies, similarly to the role of the human immunoglobulin loci in c-*myc* and *bcl*-2 deregulation in Burkitt lymphoma (*9*) and in other B-cell malignancies (*10*), respectively.

We have examined two cases of T-cell leukemias with a chromosome translocation t(8;14) involving band 14q11 and the distal long arm of chromosome 8. Figure 1A

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Table 1. Human genes in DeF-BW5147 cell hybrids.

Cell lines	*mos*	*myc*	IgH	C_α	V_α	NP	Human chromosomes*				Human c-*myc* transcripts
							8	8q+	14	14q−	
DeF	+	+	+	+	+	+	++	++	++	++	+++
BW5147	−	−	−	−	−	−	−	−	−	−	−
563 BC5	+	+	+	+	+	+	+	−	++	++	−
563 BB4	+	+	+	+	+	+	±	−	++	+	ND
563 BD3	+	+	−	−	+	+	+	−	−	++	−
563 AA1	+	+	−	−	+	+	+	−	−	+	ND
563 BB2	+	+	+	+	−	−	−	+	−	−	ND
563 BD2	−	−	−	−	+	+	−	−	−	++	ND
563 BA5-BC10	+	+	+	+	−	−	−	++	−	−	++
563 BA5-BB3	+	+	+	+	−	−	ND†	ND	ND	ND	ND
563 BA5-DE7	+	+	+	+	+	+	±	+	+	++	ND
563 AC3	+	+	+	+	+	+	−	+	−	++	+

*Percentages of metaphases containing the relevant human chromosome. A minimum of 18 metaphases of each hybrid were examined. −, none; ±, 1 to 10%; +, 10 to 30%; ++, >30%. †ND, not done. For human c-*myc* transcripts +'s and −'s were determined on the basis of analysis of the gel in Fig. 4.