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INTERLEUKIN-2 RESTORES NATURAL KILLER ACTIVITY INHIBITED BY SERA FROM HIV+ HEMOPHILIC PATIENTS

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Abstract Natural killer (NK) activity is impaired in patients with positive serology for the human immunedeficiency virus (HIV). We previously found an inhibitory effect of sera from hemophilic (He) HIV+ patients on normal NK activity. In the present study, we have further characterized this effect by studying its reversibility, temperature and time incubation dependence. Since interleukin 2 (IL-2) is able to enhance NK levels, we analized the capacity of this lymphokine to reverse the effect of He HIV+ sera. We found that when IL-2 activation of NK activity occurred simultaneously or after HIV+ serum-treatment, a significant restoration of NK function was observed. In contrast, preincubation with IL-2 did not affect the inhibitory effect exerted by HIV+ sera.

Resumen ***La interleuquina 2 (IL-2) restaura la actividad* natural killer *inhibida por sueros de pacientes hemofílicos HIV+***. La actividad *natural killer* (NK) está afectada en pacientes con serología positiva para el virus de la inmunodeficiencia humana (HIV). En trabajos previos, encontramos un efecto inhibitorio de los sueros de pacientes hemofílicos (He) HIV+ sobre la actividad NK normal. Aquí, caracterizamos este efecto estudiando su reversibilidad, así como la dependencia de la temperatura y el tiempo de incubación. Dado que la interleuquina 2 (IL-2) es capaz de aumentar los niveles de NK, analizamos la capacidad de esta linfoquina para revertir el efecto de los sueros de He HIV+. Se demostró que cuando la activación de la actividad NK con IL-2 ocurría simultáneamente o después del tratamiento con suero HIV+, se observaba una significativa recuperación de la función NK. En contraste, la preincubación con IL-2 no cambió el efecto inhibitorio ejercido por sueros HIV+.

Key words: natural killer cytotoxicity, HIV+ serum, interleukin 2, hemophilia

A profound defect in cellular immune functions is associated with an infection by the human immune deficiency virus (HIV)[1, 2]. Abnormal T lymphocyte immunity, macrophage function, natural killer (NK) cytotoxicity and pathological B cell activity have been reported[3, 6]. HIV positive hemophilic patients (He HIV+) under treatment with concentrated factor VIII frequently have impaired cellular and humoral immunity[7].

The presence of different factors against allogeneic T or B lymphocytes has been described in AIDS and He HIV+ patients[8, 10]. Such factors could alter the physiological behaviour of leukocytes. In a previous report, we have shown that IgG fractions of AIDS sera and circulating immune complexes could inhibit NK cytotoxicity by interfering both at the effector and target cell levels[11]. In this study, we have further characterized the inhibitory effect of these sera by evaluating their reversibility, temperature and time dependence. We have also analized the ability of IL-2 to reverse HIV+ serum inhibition of normal NK activity.

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Materials and Methods

Effector cells

Human peripheral blood mononuclear cells (PBMC) were prepared by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) centrifugation. Cells from the interphase of the gradients were washed three times and resuspended at a concentration of 2 x 10^6/ml in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Gibco, Grand Island, NY) and 50 µg/ml gentamycin (complete medium, CM).

Serum

Venous blood was collected from normal seronegative donors and He HIV+ patients classified according to the staging system proposed by the Center for Disease Control, as groups IVC2 and AIDS. After clotting at room temperature, it was centrifuged at 400 xg for 10 min. The serum was removed, and heat-inactivated (56°C for 30 min). Then it was centrifuged at 7 800 xg 10 min, the supernatant serum was aliquotized and stored at -70°C until use.

Reagents

Lectin-free purified interleukin-2 (IL-2) was purchased from Electronucleonics (MD, USA). Human recombinant interleukin-2 (rIl-2) and human recombinant interferon gamma (rIFN-γ) were purchased from Genzyme (Cambridge, MA, USA). All the reagents were maintained at -70°C until use.

PBMC treatment

PBMC (5 x 10^6/ml) suspended in CM were incubated with Normal (N) and HeHIV⁺ serum 1/20 during 4 or 18 h at 37°C. On the other hand, PBMC were stimulated with IL-2 (100 U/ml) or rIFN-γ (100 U/ml) for 18 h before, simultaneously or after serum addition. After each incubation cells were washed twice, adjusted to 2 x 10^6/ml and used as effector cells in the cytotoxic assay.

Target cells

The K_{562} erythroleukemia target cell line was used to measure spontaneous NK activity. It was maintained in continuous culture in CM at 37°C in a 5% CO_2 air atmosphere.

NK Cytotoxic Assay

A conventional 4-hour ^{51}Cr release assay was performed as described elsewhere. Briefly, 5 x 10^3 ^{51}Cr-labeled K_{562} target cells were mixed with PBMC (2 x 10^5), the assays were performed in quatriplicate and a final volume of 200 µl and placed in 96-well round-bottom microtiter plates (Corning, NY). After incubation at 37°C for 4 h, plates were centrifuged at 400 xg for 10 min and 50 µl of supernatant was carefully removed and counted in a gamma counter. The percent of specific lysis was calculated as follows:

$$\% \text{ NK cytotoxicity} = \frac{\text{experimental cpm - spontaneous cpm}}{\text{total cpm - spontaneous cpm}} \times 100$$

Spontaneous release did not exceed 10%.
The percentage of inhibition was calculated as follows:

$$\% \text{ NK inhibition} = \frac{\text{\% lysis PBMC control} - \text{\% lysis PBMC treated with serum}}{\text{\% lysis PBMC control}} \times 100$$

Statistical analysis

Data were analyzed using Wilcoxon rank test and $p < 0.05$ was taken as statistically significant. The results are expressed as the mean ± SD of the quatriplicate of a representative experiment.

Results

Inhibition of NK activity by HIV⁺ sera

We have previously reported that HIV⁺ sera strongly inhibit NK cytotoxicity of normal PBMC in comparison with normal sera[11]. The results in Figure 1A show that the inhibitory effect of HIV⁺ sera was not reversed by washing the cells after sera treatment and in Figure 1B it is shown that this inhibition was significantly augmented upon longer incubation times (18 h). As shown in Figure 2, the

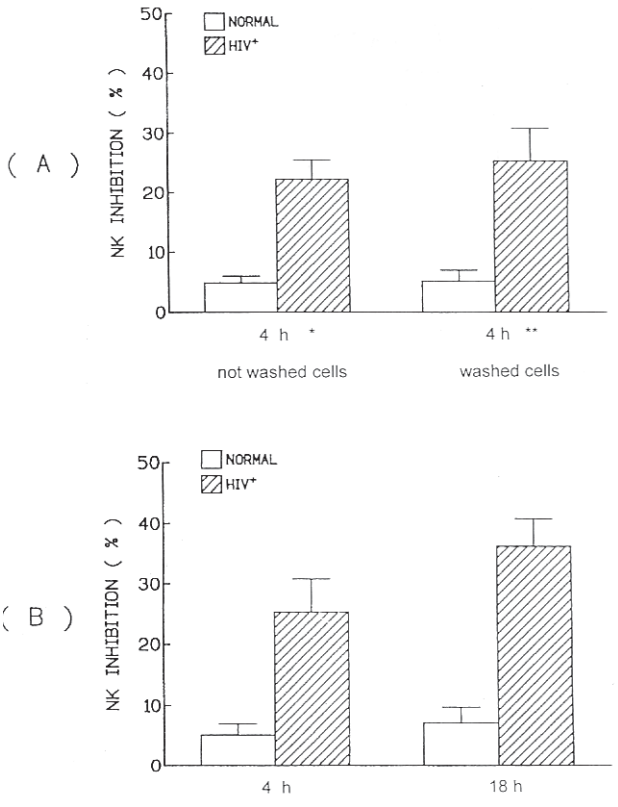

Fig. 1.– Normal PBMC (1 x 10^6) were incubated with sera from N (n = 33) or He HIV⁺ patients (n = 21). **(A)** PBMC were incubated with sera during 4 h at 37°C. After this treatment, cells were washed or not with RPMI and then were resuspended in CM. NK cytotoxicity was carried out as described in Material and Methods. Statistical significance: HIV⁺ not washed vs HIV⁺ washed: $p = 0.59$ (NS). **(B)** PBMC were incubated with sera for 4 or 18 h at 37°C, then washed with RPMI and used as effector cells in NK assay. Statistical significance: HIV⁺ 4 h vs HIV⁺ 18 h; $p = 0.016$.

inhibitory effect was also observed when PBMC were incubated with sera at 4°C; however the inhibition was lower than at 37°C, with significant differences after 4 or 18 h incubation.

Effect of IL 2 on serum inhibition of NK activity

We then investigated the possibility of reverting HIV⁺ sera effect. As IL-2 is well known to enhance NK activity of normal PBMC upon short periods of culture[12, 13], we examined whether the treatment of normal cells with IL-2 could prevent the inhibition exerted by HIV⁺ sera. As shown in Figure 3 A), B) and C), normal sera did not modify NK levels when added before, after or simultaneously with IL-2. On the contrary, treatment of effector cells with IL-2, previous to the addition of HIV⁺ sera, was unable to prevent NK inhibition (Fig. 3 D). On the other hand, treatment with IL-2 after incubation with serum markedly re-

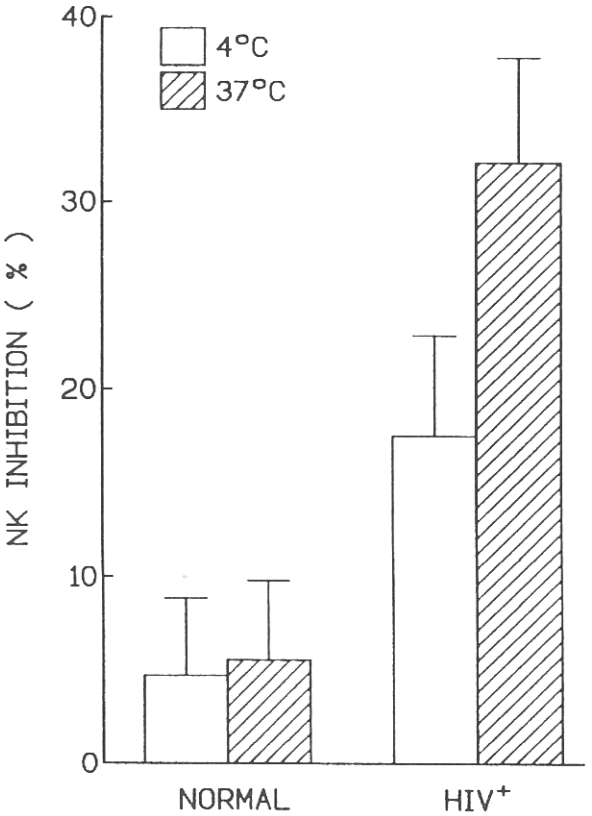

Fig. 2.– Normal PBMC (1 x 10^6) were incubated with sera from N (n = 7) or HIV⁺ patients (n = 12) during 18 h at 4 or 37°C, and then washed in RPMI. Statistical significance: HIV⁺ 4°C vs HIV⁺ 37°C: $p = 0.03$.

stored NK activity levels (Fig. 3 E). This restoration was also observed when PBMC were simultaneously incubated in the presence of IL-2 and serum (Fig. 3 F). These results were confirmed by using human recombinant IL-2; in contrast recombinant IFN gamma did not reverse NK inhibition by HIV⁺ sera (data not shown).

Discussion

During HIV infection several immunological abnormalities have been reported, including impaired NK activity[14, 15]. The NK cell activity in PBMC from AIDS patients was significantly lower than that in PBMC from both HIV-seronegative and asymptomatic subjects[16]. Hemophilic patients, as a group considered at high risk of developing AIDS, present deficient NK activity[7].

We have previously studied the regulation of NK function of He patients with positive (HIV⁺) or negative (HIV-) serology for HIV and our results indicated that both IL-2 and IFN-γ were able to restore NK levels[7, 17]. As mentioned before, in a previous study we found an inhibitory effect of sera from He HIV⁺ patients upon normal NK activity[11].

In this study, we have further characterized HIV⁺ sera inhibition. We have demonstrated that HIV⁺ serum inhibition of NK cells was not reversed by washing the cells (Fig. 1A) and it was augmented upon longer incubation times (Fig. 1B). We also found that this effect appears to be temperature dependent (Fig. 2). These findings sug-

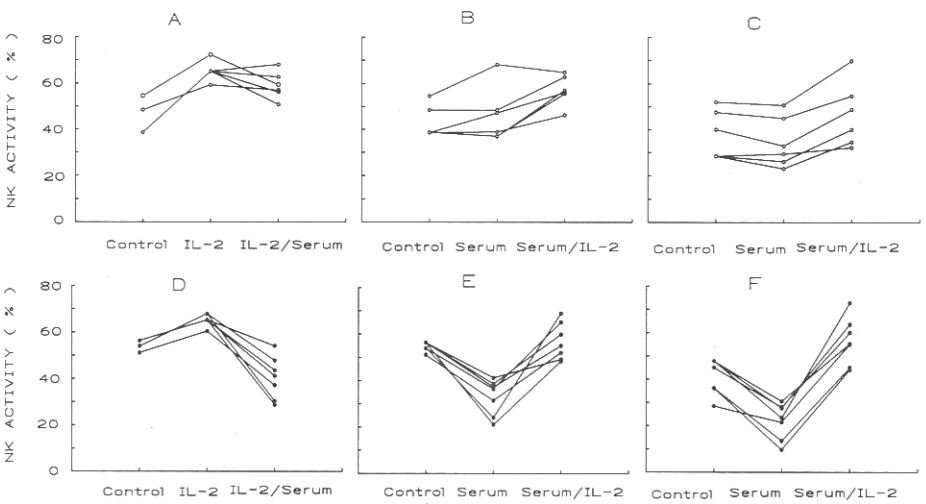

Fig. 3.– Normal NK activity was determined by the classical 4 h NK assay, as described before. Normal PBMC were incubated with sera from n ❍ (n = 6) or ● HIV⁺ (n = 7). Different incubation treatments are indicated at the bottom of the graphics: **A**) and **D**) normal PBMC were first treated with IL-2 for 18 h at 37°C, then were washed and incubated with serum (IL-2/Serum) or with CM (IL-2) for 4 h at 37°C; **B**) and **E**) normal PBMC were first incubated with sera for 4 h at 37°C. After washing the cells were treated with or without IL-2 (Serum/IL-2) or with CM (Serum) for 18 h; **C**) and **F**) normal PBMC were incubated with serum (Serum) or simultaneously with Serum and IL-2 (Serum + IL-2) during 18 h at 37°C. After these treatments cells were washed and used as effector cells in NK reaction. NK cytotoxicity of PBMC: 41.9 ± 3.0; NK cytotoxicity in the presence of IL-2: 59.4 ± 4.2. Statistical significance: HIV⁺ serum vs IL-2/HIV⁺ serum: $p = 0.76$; HIV⁺ serum vs HIV⁺ serum/IL-2: $p = 0.007$; HIV⁺ serum vs HIV⁺ serum + IL.2: $p = 0.0009$.

gest that intracellular metabolic events determine the outcome of the inhibitory effect.

Serum factors could contribute to the previously described impairment of NK activity during HIV infection[11] since these cells are exposed in vivo to the persistent inhibitory effect of these factors. At the present time, there is no clear evidence of the nature of the serum factors involved in the inhibiton of NK activity. According to our previous results[11], this inhibition could be the consequence of a direct interaction of immunoglublin G (IgG) with effector NK cells. Several investigators have reported the presence of antilymphocyte antibodies in sera of AIDS/LAS and He patients[9, 18, 19]. Antibodies to NK cells were also detected in samples from HIV-infected patients, although the potential role of these antibodies remains unknown[20]. An immunosuppressive factor was identified and partially purified from sera of AIDS patients. This factor shares epitopes with the gp41 viral transmembrane component of HIV-1 and profoundly inhibits normal T lymphocyte and NK functions[21]. HIV-gp41 is a potent modulator of cytokine production by PBMC, by increasing IL-10 secretion from normal monocytes/macrophages and down-regulating IL-2 and IFN gamma[22].

We then investigated the capacity of IL-2 to reverse HIV⁺ sera effect, demonstrating that IL-2 treatment could interfere with sera inhibition of NK activity only under two situations: 1) when IL-2 stimulation of PBMC was carried out simultaneously with sera incubation and 2) when sera treatment of PBMC preceded IL-2 activation (Fig. 3).

It has also been reported that anti-IL-2 antibodies are frequently encountered in HIV⁺ sera[23]. Therefore, one explanation for the restorative action of exogenous IL-2 would be that it helps to overcome depletion of this lymphokine by anti-IL-2 antibodies.

In this study, IL-2 activated cells were unable to overcome sera inhibition upon NK activity. There data correlate with a report[24] in which it was demonstrated that Fcγ RIIIA ligands, under the form of immune com-plexes or anti CD16 monoclonal antibody, inhibit IL-2 induced NK cell proliferation, which means that IL-2 preactivation is required in order for the FcγR ligands to mediate an antiproliferative effects.

We can conclude that IL-2 reverses sera inhibition of NK activity when it acts as a second signal, that is, when cells are IL-2 stimulated simultaneously or after sera treatment, but that there is no reversion of the sera inhibition effect when IL-2 acts as a first signal. Since HIV⁺ serum factors could aggravate the functional defect of NK cells in HIV infection it is important to consider the role of IL-2 in the restoration of NK function.

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[...] There would be no point in writing long and bad tempered protests against relativism if it were merely the doctrine that there is no such thing as knowledge: we could dismiss it instantly by asking the question, How do you know? But we can also make relativism into something passably intelligent, namely an attempt to explain how we can have reliable knowledge, given that our thinking is shaped, willy nilly, by the particular languages we happen to speak, or the taxonomies and measuring systems we have inherited with them. The goal of intelligent relativism would be to stop us daydreaming about a utopia of immaculate knowledge, and persuade us to settle for such partial certainties as the human condition allows. Clearly relativism in this sense is a gentle encouragement to scientific endeavour, not a counsel of epistemological despair.

[...] No tendría sentido escribir largas y malhumoradas protestas contra el relativismo si este fuera meramente la doctrina de que no hay tal cosa como el conocimiento: podríamos descartarla inmediatamente preguntando ¿Y usted, cómo lo sabe? Pero también podemos hacer del relativismo algo pasablemente inteligente, un intento de explicar como podemos tener un conocimiento confiable, dado que nuestro pensamiento está formado, sea como sea, por los particulares lenguajes que hablamos, o las taxonomías y sistemas de medida que hemos heredado con ellos. La meta del relativismo inteligente sería detener nuestro ensueño de una utopía de conocimiento inmaculado y persuadirnos a aceptar las parciales certezas que la condición humana nos permite. Claramente el relativismo en este sentido es un amable aliento al empeño científico y no un consejo de desesperación epistemológica.

Jonathan Rée

Crude weapons of sciences friend's. Nature 1998; 396: 31-33. Comentario de *A House Built on Sand: Exposing Postmodernist Myths about Science,* editado por Noretta Koertge. Oxford: Oxford University Press, 1998, p 322