CTROL. Para el grupo tratado con IFREC, que fue aleatorizado con el CTROL, este resultado es incuestionable y concuerda con el ya reportado de retraso en la progresión a la enfermedad. En los grupos IFLEU y FT cabe la duda de que no fueron producto de una distribución aleatoria con el control con el que se compara. En el grupo FT, hay además una diferencia en el criterio de inclusión. Sin embargo, el grupo CTROL fue concurrente con los anteriores la mayor parte del tiempo que duró la prueba, por lo que la comparación tiene cierta validez y las diferencias encontradas son considerables, por lo que deben tenerse en cuenta.

IMMUNOENZYMATIC ASSAY FOR THE QUANTIFICATION OF RECOMBINANT HUMAN INTERLEUKIN-2

Giselle Pentón, Francisco Hernández*, Vivian Santos, Yolanda Rojas, Eduardo Pentón, Pedro López-Saura and Manuel Araña.

Genetic Engineering and Biotechnology Center, P.O. Box 6162, La Habana 6. C.P. 10600, Cuba.

INTRODUCTION

In the present paper an immunoenzymatic assay was developed for determination of recombinant human IL-2 concentrations in the range of nanomoles based upon the use of murine monoclonal antibodies (MAbs) developed and specially selected for this purpose. The system was calibrated against an international standard of IL-2 and the calculation of concentrations of samples was optimized through the use of a log-log transformation which allowed the use of the calibration curve in the concentration range as low as 1 ng/mL with a regression coefficient higher than 0.99.

EXPERIMENTAL PROCEDURES

The obtainment of MAbs was achieved according to a variant of the conventional method of Köhler and Milstein (1). Immunoglobulins were conjugated with radish peroxi lase by the peryodate method (2).

The adequate concentrations and times of incubation were evaluated using polyvinyl plates coated with 1; 5; 10; 20; 50 and 100 μg/mL of the coating MAb in different conditions including: overnight incubation at 4°C, 3 h at 37°C and preincubation 2 h at 37°C followed by overnight incubation at 4°C.

The plates were incubated with the antigen, conjugated antibody and after adding substrated and stopped the reaction, the reading was performed in an ultramicroanalytical system (SUMA).

In the same way the optimal time and temperature conditions of incubation with the antigen using three times (1, 2 and 3 h) and three temperatures (25, 37 and 42°C) were evaluated. The sensitivity of the system was determined using the absorbance values of negative controls and the three more diluted points of the standard curve of 20 determinations. Concentrations of IL-2 were calculated using a log-log transformation.

RESULTS AND DISCUSSION

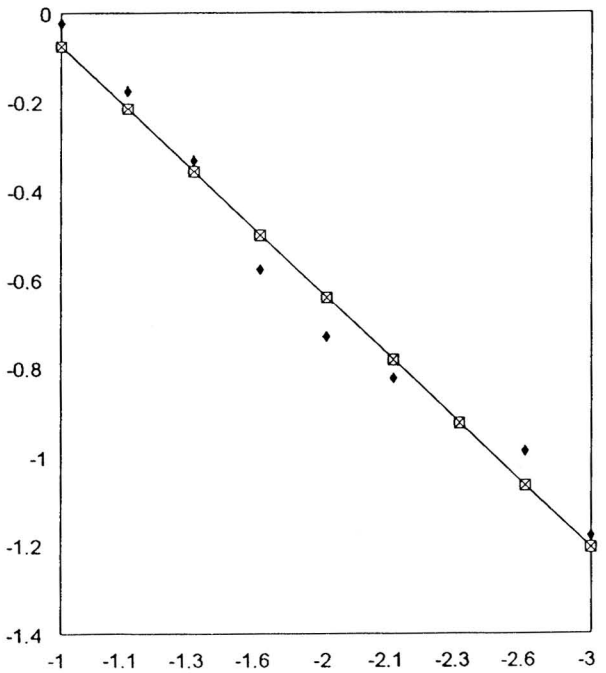
In our ELISA system the optimal conditions obtained were: overnight coating at 4°C and 10 μg/mL of the MAb CBIL-2.2, polyvinyl as solid phase and incubation during 1 h at 37°C with the antigen. The coating of plates has been shown to be optimal at certain concentration values under which it is less efficient (3).

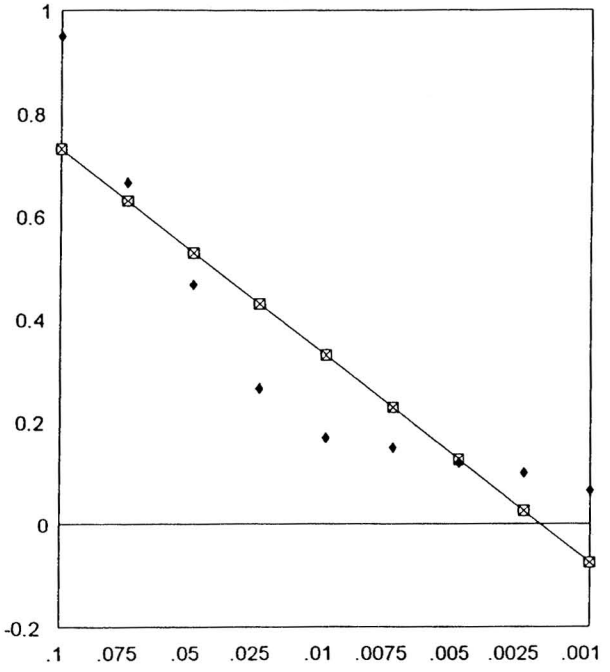
In our system 10 μg/mL was the adequate coating concentrations which does not differ significantly from the higher concentrations employed but it does from the 1 and 5 μg/mL concentrations. By the other hand the overnight coating at 4°C was enough for the binding to the solid phase of all the capture antibody. The incubation during 1 h at 37°C with the antigen resulted the best condition assayed which was evidenced by an increase of the slope of the regression line at this temperature.

The sensitivity of the system was 1 ng/mL which corresponded to 10 units of IL-2 per milliliter as determined by the demonstration of a significant difference between the absorbance values of negative controls and the point of the standard curve corresponding to 1 ng/mL of IL-2.

A log-log transformation was employed as shown in the following picture which allowed to increase the correlation coefficient from 0.90 to 0.99.

**Should be also considered as first author*





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THE IFNγ PATHOPHYSIOLOGY. THE ROLE OF SOLUBLE IFNγR α CHAIN

Yraldo Bello[1], A. Martinto[1], Marisela Suárez[1], Giselle Pentón[1], Isabel Apezteguía[1], O. Fernández[1], Alfredo Menéndez[2]; Michel Aguet[3] and Pedro López Saura[1].

[1]Department of Cellular Biology. Center for Biological Research, La Habana 6, P.O.Box 6996. [2]Genetic Engineering and Biotechnology, La Habana 6, P.O. Box 6162. C.P. 10600, Cuba. [3]Molecular Biology I. Univ. Zurich, Honggerberg, Zurich 8093, Switzerland.

INTRODUCTION

IFNγ is a lymphokine produced by T cells and NK cells. IFNγ exerts its activities by binding to specific cell surface receptor (R).

IFNγ acts as a potent immunomodulator and is a powerful macrophage activating factor. In the basis of its properties the IFNγ could play the principal role in the antigen specific immune response and has been considered the master key to the inflammatory response (1).

Some experimental findings point to a disease-promoting role of IFNγ in several pathological status as multiple sclerosis, systemic lupus erithematosus, type I diabetes, septic shock and others (2). In the case of rheumatoid arthritis (RA) specially the route of administration are of critical importance in determining the effects of IFNγ.

It is possible that at sites of inflammation the pro-inflammatory properties of IFNγ predominate, whereas critical concentration of circulating IFNγ are anti-inflammatory.

An aberrant regulation of the IFNγ action at site of inflammation could contribute to the development and/or exacerbation of some autoimmune and inflammatory disorders.The control of IFNγ function at the