CD8+/CD38+: immune activity and clinical significance in HCV patients with and without interferon therapy

A. PERRELLA, O. PERRELLA*, P. CONCA, G. TARANTINO

Department of Clinical and Experimental Medicine, Federico II University Medical School - Naples (Italy)
*VII Department of Infectious Diseases and Immunology, D. Cotugno Hospital - Naples (Italy)

Abstract. – At the light of the importance of cytotoxic T lymphocyte (CTL) response during chronic hepatitis C, we carried out a study in order to evaluate the CD8+/CD38+T-cells, immunophenotypic marker of CD8+ activated cells in a selected cohort of 22 patients for four months. The patients were subdivided in two groups: A (with IFN therapy), B (without IFN therapy). The results show that in IFN-treated subjects there is a significant reduction of ALT (sign test, $z = .424; p < .05$) and that the CD8+/CD38+ present a positive correlation with HCV-RNA ($r = .894; p < .05$). We hypothesize that during IFN therapy the CD8+/CD38+ activity is able to oppose HCV, probably by increasing MHC I expression on the infected cells due to the IFN modulatory action, that could strengthen the immune response of CD8+ activated T-lymphocytes. These events confer the capacity to specifically respond to any viral replication and probably take part in the reduction of ALT levels by decreasing the chronic inflammation present during a defective immune response. These data show think CD8+/CD38+ marker could be a good parameter to evaluate both the viral activity and immunological status in HCV+ patients undergoing IFN treatment.

Key Words:

HCV, IFN, CTL, Immunophenotypes, MHC, Cytometry.

Introduction

Observing the natural history of chronic hepatitis C (CHC), it is evident that in the 80-85% of HCV+ patients (pts) it is the evolution of an acute episode of hepatitis C[1,2]. The immune mechanisms implicated in the viral persistence and in the progressive liver impairment are so far poorly understood. Nevertheless, at present it seems that cytotoxic T-lymphocyte (CTL) activity and Th1/Th2 balance play an important role in the evolution of the disease during the acute and chronic phases of the infection[3,4]. In particular, in the complex immune network ongoing during the infection, the CD8+ cytotoxic T-lymphocyte's role seems to be decisive for the subsequent progress of the disease, according to many investigations[5,6,3]. Moreover, it has been demonstrated that early escape from immunodominant CTL responses may be an important factor of HCV persistence[7,5], while increased cytotoxic T-cells activity is associated with the viral clearance[8]. On the basis of these evidences, we hypothesized that the activated CD8+ T-lymphocytes (CD8+/CD38+) evaluation, in patients with and without IFN treatment, could be useful to better understand the ongoing immunopathological events and could represent a screenig tool of the cytotoxic immune response. The considered marker (CD38+) is surface protein present on activated CD8+ lymphocytes (CTL) versus the antigen (Ag) present during infection[9]. We considered measuring this parameter, because it had previously been performed in HIV infected pts[9] to check cytotoxic T-cells activity and had shown a good accuracy.

In this contest, to verify the action of α-Interferon (IFN) on CD8+/CD38+, we decided to evaluate two sets of parameters, i.e., cytolysis and viral replication, strictly related to this T-cells subset, in order to observe possible modifications, by associating it to HCV serum concentration and ALT levels.

Materials and Methods

Study design

In our pilot investigation, we enrolled 22 naive patients with histologically proven CHC (mostly genotype 1b). HCV-RNA, ALT and CD8+/CD38+ on peripheral blood were measured in two different times (initial time, T0; and final time, after four months, T4). After the first sampling, the selected cohort of patients was subdivided into two well-matched (age and sex) groups of 11 pts each: group A, was treated with IFN therapy and group B without IFN. At T0 sampling, both groups were IFN-free. The chosen schedule for IFN dosage was 3 MU/daily of α-2b recombinant IFN for the whole study period.

Immunological, viral and biochemical analysis

The examination of immunophenotypic marker, after T cell counts, was performed by flow cytometry as previously described[10]. The cells were incubated with 10 mL of monoclonal antibodies (MoAbs) conjugated with PE and FITC by using a single step procedure in PBS enriched with 0.5% BSA at 4° C for 20 minutes. The following MoAbs were used: CD8-PE AND CD38-FITC (Ortho Diagnostic) according to a standard procedure. The T lymphocytes value was expressed in percentage.

The HCV-RNA measurement in sera was performed by means of a prototype branched DNA (bDNA) signal amplification assay (Chiron, Emeruyville, CA). The hybridised and immobilized complex of viral RNA, was linked to phosphatase alkaline (bDNA) probe and incubated with chemiluminescent substrate. Light emission was measured and the quantity was determined from standard curve, constructed in each run from standards containing known amounts of synthetic HCV RNA. The lower detection limit was of 200 copies/mL. Serum ALT determination was performed by our centralized laboratory, setting the normal values at < 40 U/L.

Statistics

For the true analysis, we preferred to use nonparametric tests, i.e., Sign test for the evaluation of the dependent samples and Mann-Withney test in estimating the independent ones. On the contrary, when managing correlations, to obtain a more robust assessment, the Pearson's coefficient was applied. The SPSS package (version 9 for Windows) was utilized.

Results

The T0 results were characterized, on measuring association (Spearman's r), by no significant correlation amongst chosen parameters. At T4 performance, the experimental data displayed that in the patients undergoing IFN therapy (group A) there was a statistically significant ALT reduction (sign test, $z = .424$; $p < .05$) (Figure 1) compared to the T0 levels, while no noteworthy fluctuation of the viremia was present. Moreover, the same group displayed the presence of positive correlation between CD8+/CD38+ T-lymphocytes and HCV-RNA ($r = .814$; $p < .05$) (Table I). On the contrary, the patients without therapy (group B) did not exhibit any significant difference in comparison with T0 values and no evident correlation. As further

Table I. The table shows the levels of significancy ($p < 0.05$) of the studied parameters correlations.

Spearman's Rho		RNA.T4	CD8+/CD38+	ALT.T4
RNAT4	Correlation	1.000 –	.814* .014	.599 .117
CD8+/CD38+	Correlation	.814* .014	1.000 –	.333 .381
ALT.T4	Correlation	.599 .117	.333 .381	1.000 ,

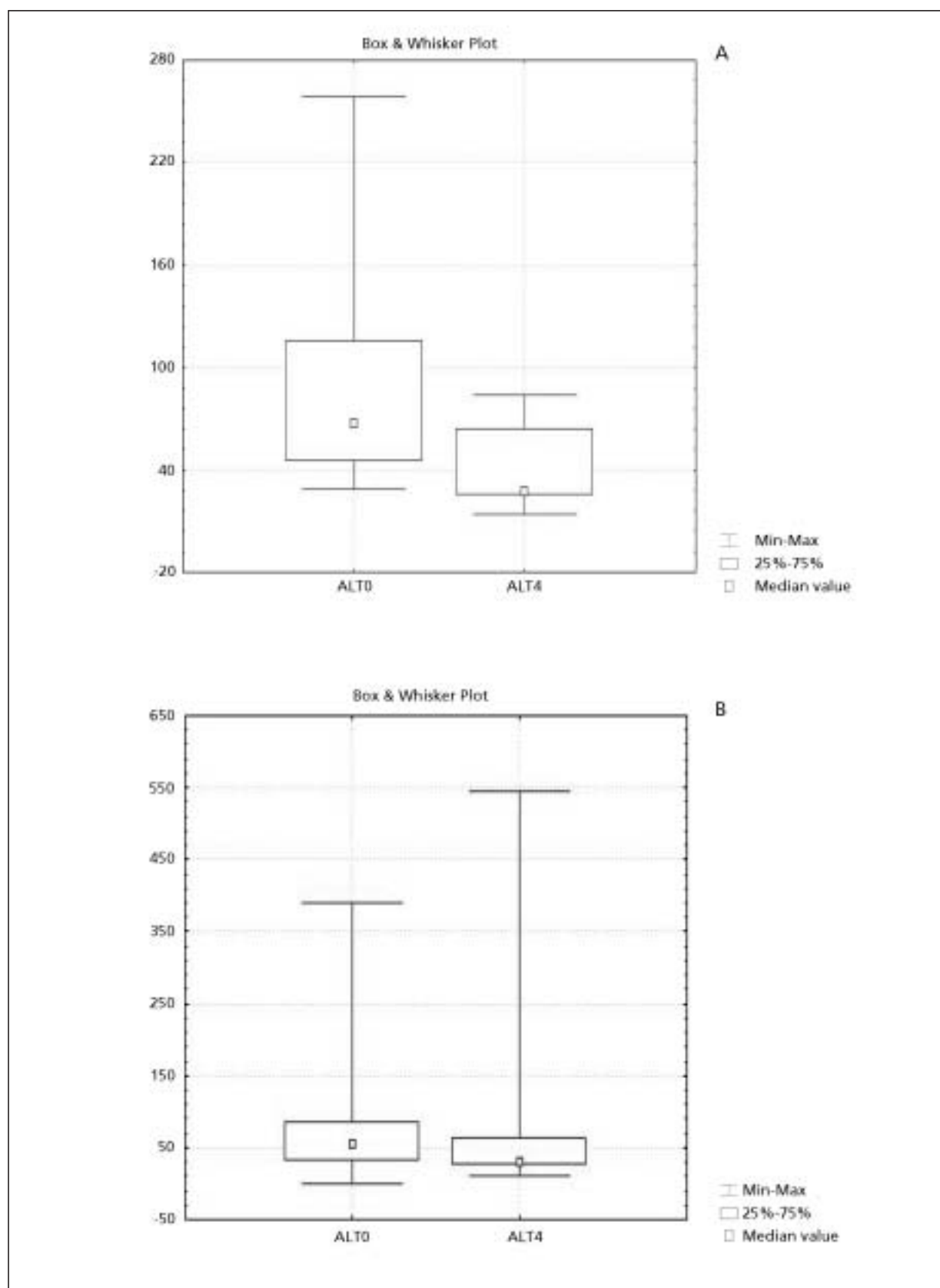*Displays the statistically significant correlations.

Figure 1. *A*, Patients with IFN therapy. *B*, Patients without IFN therapy.

analysis, the U Mann-Whitney test did not show any significant change in CD8+/CD38+ values between groups A and B.

Discussion

These results suggest that during IFN therapy even though there was no significant decrease of viremia levels, nor a difference in CD8+/CD38+ values, we obtained the ALT reduction. Moreover, a variation of viral load and replicative cycle corresponded to a change in the same sense of CD8+ activation, as showed in correlation Table. On the ground of our data, considering that there was no difference between the two groups as to the CD8+/CD38+ concentration, we could hypothesize that the action of IFN on CD8+ probably takes effect by means of an increased expression of MHC class I, reinforcing the CD8+/CD38+ activity against HCV. So, in this particular context of immunostimulation, this one T-lymphocyte subset could carry on its role in the immune response against the infected cells at any viral replication increase, resulting more specific in the host defence. The CD8+/CD38+ T-lymphocytes, thanks to IFN action, could be probably more vigorous and able to freeze and prevent the viral replication strategy, because of a strengthening of immune specific mechanisms[11], like apoptosis, that result to be less harmful for liver, because it induces a lower inflammatory response and consequently fibrosis. All these events, avoiding a chronic inflammatory activity owing to a defective liver resident T-cells immune response in CHC[12], could have a role in determining a significant ALT reduction in association with IFN-dependent antiviral modulation. On the contrary, the patients, that were not treated with IFN, result to maintain the incomplete immune cytotoxic defective response, due to the immuno-defective Th1 activity, occurring during the first phase of disease. In this group, the cytotoxic activity results not to be strong enough to oppose and prevent the viral activity; this event in association with HCV immunological escape mechanism is probably the cause of the higher ALT levels and determines the absence of some kind immune response against the HCV, as HCV-RNA correlations show.

The IFN therapy can be still a good pharmacological treatment to improve the CD8+/CD38+ response, even thought the long-term effects on CTL should be still proved during the natural history of the chronical disease.

In conclusion, we could, speculate that the immune balance during the IFN treatment in CHC patients results to be favourable toward the CD8+/CD38+ immune response and evolution, refraining from the viral escape.

In the course of IFN therapy the CD8+/CD38+ T-lymphocytes result to be able to mount an adequate immune response against the virus and the measurement of this ratio could be an useful tool to assess immune cytotoxic activity in relation to the viral concentration, differently from what is seen in untreated patients. This marker therefore could, in future, represent a reliable and inexpensive instrument to estimate cytotoxic and viral activities in patients treated with IFN therapy, but further investigations are required to better understand the role of CD8+/CD38+ during CHC and the implication of this T-cells population.

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