

CD3- and CD28-Activating Pathways in HCV Infection

JOSÉ A. CORADO, FELIX I. TORO, MIREN L. BAROJA, NICOLAS E. BIANCO,
and IRMA V. MACHADO

ABSTRACT

We investigated the proliferative response and IL-2 receptor (IL-2R) expression in peripheral blood mononuclear cells (PBMC) activated with anti-CD3 mAb alone or in combination with anti-CD28 mAb in a group of hepatitis C virus (HCV)-infected patients with detectable viremia demonstrated by "nested" PCR. PBMC from HCV patients and controls showed similar proliferative responses either to anti-CD3 mAb, 64.1, and/or to anti-CD28 mAb, 9.3. No differences were found in anti-CD3 or anti-CD3 plus anti-CD28-induced proliferative responses between patients who demonstrated circulating PBMC bearing HCV-RNA when compared to those with negative HCV-RNA PBMC. Moreover, flow cytometry studies confirmed that anti-CD3 alone or in combination with anti-CD28 were able to induce a significant increase of IL-2R expression in patients or controls PBMC. Both groups showed similar basal CD28 expression. These results indicate that both CD3- and CD28-activating pathways are preserved in HCV-infected patients with chronic active liver disease.

Hepatitis C virus (HCV) as hepatitis B virus (HBV) induces chronic liver disease (5). However, in contrast to HBV infection, the hypothesis that host immunological alterations might be associated with the clinical outcome of hepatitis C awaits further demonstration (14). In T-cell-mediated immune responses, the interaction between the T-cell antigen receptor (TCR/CD3) complex with a putative antigen is complemented by antigen-presenting cells that provide essential signals for T-cell activation (4). This process can be mimicked *in vitro* utilizing monoclonal antibodies (mAbs) directed against the CD3 antigen (12). Interactions with other accessory T-cell surface molecules are also necessary for a full immune response (16). For instance, the CD28 antigen amplifies the responses of antigen and mitogen-activated T cells (1,10,11). We investigated the immune response of patients with chronic HCV infection using peripheral blood mononuclear cells (PBMC) that were activated through the CD3 and CD28 surface molecules. Fourteen patients, mean age 39 ± 14 years, with the diagnosis of chronic HCV infection were selected. They showed an alanine-aminotransferase (ALT) activity 3.5-fold above the normal upper limit and repeatedly positive antibodies to HCV (anti-HCV) (2nd generation, Abbott Laboratories, North Chicago, IL) at least three times before being admitted to the study. All patients were also positive for HCV RNA in serum as tested by nested PCR according to the method of Inchauspe

et al. (8) and 70% of them were tested for HCV RNA in PBMC. All patients were studied before interferon- α 2a therapy; 4 of them were reevaluated after 6 months of therapy (Roferon A®, F. Hoffmann-LaRoche, Basel, Switzerland). Fourteen seronegative healthy individuals were used as controls. PBMC were isolated from heparinized blood using Ficoll-Hypaque gradient separation medium (Pharmacia Chemicals, Uppsala, Sweden). Cells (1×10^5) were cultured in the presence of optimal concentration (10 ng/ml) of anti-CD3 mAb 64.1 (Bristol Myers Squibb, Seattle, WA) and/or 50 ng/ml of anti-CD28 mAb 9.3 (Bristol Myers Squibb, Seattle, WA). The cells cultures were carried out in triplicate and incubated for 96 hr at 37°C in a 5% CO₂ atmosphere. PBMC were also cultured in the presence of 0.5 μ g/ml of phytohemmagglutinin (PHA) (Wellcome Diagnostics, Dartford, England). Cell proliferation was assessed by [³H]thymidine incorporation (New England Nuclear, Boston, MA). The expression of IL-2 receptor (IL-2R) was evaluated by direct immunofluorescence in unstimulated and stimulated PBMC as described above, using FITC-conjugated anti-IL-2R (p55) mAb (Ortho Diagnostics System, Raritan, NJ). Basal expression of CD28 on the surface of PBMC was evaluated by indirect immunofluorescence using the anti-CD28 mAb 9.3 (IgG_{2a}) (1 μ g/ml) and FITC-conjugated goat anti-mouse IgG (Becton-Dickinson, Lincoln Park, NJ). Fluorescence analysis was performed by flow cytometry (EPICS 753, Coulter Corporation, Hialeah, FL).

Figure 1 shows similar PBMC proliferative responses to anti-CD3 mAb (10 ng/ml) from HCV patients and controls. When the anti-CD28 mAb was added (50 ng/ml) in combination with the anti-CD3 mAb, a significantly higher proliferative response was observed in either patients or controls when compared to anti-CD3 alone (Fig. 1). No differences were observed between patients and controls when increasing concentrations of mAb 64.1 were used ranging between 1 and 50 ng/ml. Similarly, both groups responded equally to PHA (data not shown). When we compared the proliferative response of patients with PBMC positive for HCV RNA versus patients with PBMC negative for HCV-RNA, no difference was found (Fig. 1). Interferon- α 2a therapy did not alter the proliferative response to anti-CD3 and/or anti-CD28. Basal expression of CD28 molecule on PBMC from HCV-infected patients was similar to controls (mean \pm SD = 66.5 \pm 9.1 and 78.0 \pm 8.5%, respectively). Besides, under nonstimulated conditions, IL-2R expression in PBMC from HCV patients and controls were similar (Table 1). A clear enhancing effect on IL-2R expression was observed in PBMC stimulated with the anti-CD3 mAb 64.1 (10 ng/ml) in HCV patients and controls (Table 1). When the anti-CD28 mAb 9.3 was added at optimal concentration (50 ng/ml) to the PBMC cultured with mAb 64.1, a significant increase in the expression of IL-2R was observed in both groups when compared to that obtained with mAb 64.1 alone ($P <$

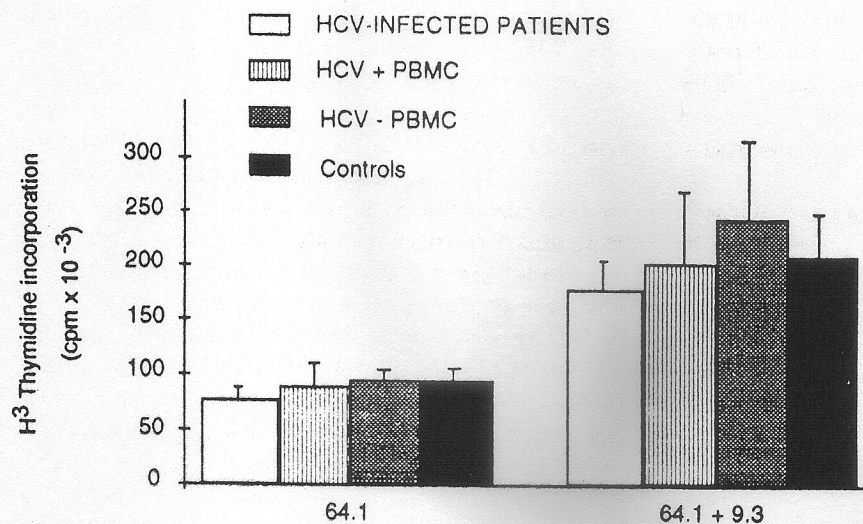


FIG. 1. Proliferative response of PBMC from HCV-infected individuals. PBMC were stimulated with mAbs 64.1 (anti-CD3) and 9.3 (anti-CD28). HCV-infected patients represent mean \pm SE of 14 patients and 14 controls. HCV (+) PBMC and HCV (-) PBMC values represent mean \pm SE of 4 patients each. In all the cases spontaneous proliferation (medium) was always below 1,000 cpm.

CD3 AND CD28 PATHWAYS IN HCV INFECTION

TABLE 1. IL-2R EXPRESSION ON PBMC FROM HCV-INFECTED PATIENTS CULTURED WITH ANTI-CD3 AND/OR ANTI-CD28^a

	Culture condition		
	NS	64.1 ^b	64.1 + 9.3 ^c
Patients	4 ± 1	30 ± 4	50 ± 5*
Controls	3 ± 1	25 ± 3	47 ± 6*

^aValues are indicated as percentage of positive cells for IL-2R expression (mean ± SD) of 9 experiments for each condition. NS, nonstimulated cells.

^bAnti-CD3 mAb, 64.1 (10 ng/ml).

^cAnti-CD-3 mAb, 64.1 (10 ng/ml) + anti-CD28 mAb, 9.3 (50 ng/ml).

* $P < 0.001$.

0.001) (Table 1). There was a significant direct correlation between PBMC proliferative responses and IL-2R expression to both stimuli in either HCV patients ($r = 0.98$) or controls ($r = 0.99$).

The factors that contribute to the persistence of liver necroinflammation in HCV chronic infection are still under investigation (7,14). The identification of several HCV strains, the existence of mutant viruses, and the possible inability of the host immune system to mount an effective response have all been suggested as contributors of chronic HCV infection (6). We investigated the lymphocyte activation function of PBMC through the CD3 and CD28 pathways in a group of HCV-infected patients with chronic hepatitis and detectable viremia. We found that the PBMC from HCV-infected patients were able to sustain proliferative responses when stimulated with both anti-CD3 and/or anti-CD28 mAbs. This response correlated directly with the expression of IL-2R. Recently, we demonstrated that PBMC from HBV chronic carriers bearing "e" antigenemia and positive HBV-DNA responded less than healthy controls when cultured with anti-CD3 mAb (2). In contrast, PBMC from patients chronically infected with HCV and detectable viremia did not show perturbation of the CD3 activation function. Furthermore, we found that the pattern of response mediated by CD3 and CD28 pathways was unaltered among patients who presented PBMC infected by HCV and patients who did not show circulating PBMC infected by HCV. This observation tends to suggest that infection by HCV of immunocompetent cells does not alter T-cell responses. Our results are in agreement with the reports of Botarelli et al. (3) and Schupper et al. (15) who concluded that the majority of patients with chronic hepatitis C showed antigen-dependent PBMC proliferative responses to both HCV structural and nonstructural proteins. Recent reports have suggested that the second signal delivered by CD28 could prevent clonal anergy *in vitro* (9). In this regard, PBMC-derived T-cell clones have been obtained by Minutello et al. from HCV-infected individuals demonstrating that these patients maintained antigen-dependent responses (13) suggesting that the CD28 pathway is indeed unaltered. In addition, HCV-infected patients showed similar expression of CD28 to those obtained in normal controls. This report adds new evidence that suggests that in HCV chronic infection the antigen-independent T-cell proliferative response is also unaltered. In addition, our results indicate that, in contrast to HBV infection, HCV-infected patients with chronic liver disease, active viremia, and irrespective of the presence of circulating mononuclear cells bearing HCV-RNA, do not exhibit abnormalities of T cells activated through CD3 and CD28 pathways.

ACKNOWLEDGMENTS

This work was supported by grants from CONICIT (Project Nos. S1-2471 and S1-2520) and Génic Program. We thank Drs. Juan De Sanctis and Isaac Blanca for helpful suggestions and critical review of the manuscript. We thank Hoffmann-LaRoche Laboratories for providing us with interferon- α 2a.

REFERENCES

1. Baroja, M.L., K. Lorre, F. Van Vaeck, and J.L. Ceuppens. 1990. The T cell monoclonal antibody 9.3 (anti-CD28) provides a helper signal and bypasses the need for accessory cells in T-cell activation with immobilized anti-CD3 and mitogens. *Cell. Immunol.* 120:205-217.
2. Baroja, M.L., F.L. Sirit, D.J. Caldera, F.I. Toro, M.E. Zabaleta, C.J. Colmenares, N.E. Bianco, and I.V. Machado. 1993. Anti-CD3-activated T-cells from chronic nonviremic HBV carriers are hyperreactive to monocytic accessory signals. *Clin. Immunol. Immunopathol.* 69:180-188.
3. Botarelli, P., M.R. Brunetto, M.A. Minutello, P. Calvo, D. Unutmaz, A.J. Weiner, Q.L. Choo, J.R. Shuster, G. Kuo, F. Bonino, M. Houghton, and S. Abrignani. 1993. T-lymphocyte response to hepatitis C virus in different clinical courses of infection. *Gastroenterology* 104:580-587.
4. De Vries, J., H. Yssel, and H. Spits. 1989. Interplay between the TCR/CD3 receptor complex and CD4 or CD8 in the activation of cytotoxic T lymphocytes. *Immunol. Rev.* 109:119-141.
5. Di Bisceglie, A.M., Z.D. Goodman, K.G. Ishak, J.H. Hoofnagle, J.J. Melpolder, and H.J. Alter. 1991. Long-term clinical and histopathological follow-up of chronic post-transfusion hepatitis. *Hepatology* 14:969-974.
6. Farci, P., H.J. Alter, S. Govindarajan, D.C. Wong, R. Engle, R.R. Lesniewski, I.K. Mushahwar, S.M. Desai, R.H. Miller, N. Ogata, and R.H. Purcell. 1992. Lack of protective immunity against reinfection with hepatitis C virus. *Science* 256:135-140.
7. Houghton, M., A. Weiner, J. Han, G. Kuo, and Q.L. Choo. 1991. Molecular biology of the hepatitis C viruses: Implications for diagnosis, development and control of viral disease. *Hepatology* 4:381-388.
8. Inchauspe, G., K. Abe, S. Zebedee, M. Nasoff, and A.M. Prince. 1991. Use of conserved sequences from hepatitis C virus for the detection of viral RNA in infected sera by polymerase chain reaction. *Hepatology* 14:595-600.
9. Harding, F.A., J.G. McArthur, J.A. Gross, D.H. Raulet, and J.P. Allison. 1992. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature (London)* 356:607-609.
10. June, C.H., J.A. Ledbetter, P.S. Linsley, and C.B. Thompson. 1990. Role of CD28 receptor in T-cell activation. *Immunol. Today* 11:211-216.
11. Linsley, P.S., and J.A. Ledbetter. 1993. The role of CD28 receptor during T cell responses to antigen. *Ann. Rev. Immunol.* 11:191-212.
12. Meuer, S.C., J.C. Hodgdon, R.E. Hussey, J.P. Protentis, S.F. Schlossman, and E.L. Reinherz. 1983. Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J. Exp. Med.* 158:988-993.
13. Minutello, M.A., P. Pileri, D. Unutmaz, S. Censini, G. Kuo, M. Houghton, M.R. Brunetto, F. Bonino, and S. Abrignani. 1993. Compartmentalization of T lymphocytes to the site of disease: Intrahepatic CD4+ T cells specific for the protein NS4 of hepatitis C virus in patients with chronic hepatitis C. *J. Exp. Med.* 178:17-25.
14. Scheuer, P.J., P. Ashrafzadeh, S. Sherlock, D. Brown, and G.M. Dusheiko. 1991. The pathology of hepatitis C. *Hepatology* 15:567-571.
15. Schupper, H.P., H.J. Scheffel, S. Aceituno, T. Paglieroni, P.V. Holland, and J.B. Zeldis. 1993. Peripheral-blood mononuclear cell responses to recombinant hepatitis C virus antigen in patients with chronic hepatitis C. *Hepatology* 18:1055-1060.
16. Van Severter, G.A., Y. Shimizu, and S. Shaw. 1991. Roles of multiple accessory molecules in T cell activation. *Curr. Opin. Immunol.* 3:294-303.

Address reprint requests to:

*Dr. José A. Corado
 Instituto de Inmunología
 Facultad de Medicina
 Universidad Central de Venezuela
 Apartado 50109
 Sabana Grande, Caracas 1050-A, Venezuela*