may be the case with regard to the development of *BCR/ABL* and t(9;22).

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Replication of Hepatitis C Virus in B Lymphocytes ($CD19^+$)

To the Editor:

In recent years, the presence of hepatitis C virus (HCV) genomic sequences (plus-strand) and replicative intermediate (minus-strand) in the peripheral blood mononuclear cells (PBMC) has been reported.[1] To clarify which PBMC subpopulation is infected, and to evaluate active production of viral particles in target cells, we searched for the presence of viral genomic and antigenomic-RNA in PBMC and PBMC subset of 4 HCV chronically infected patients, before and after mitogenic stimulations of cells.

Peripheral blood mononuclear cell subpopulations ($CD4^+$, $CD8^+$, $CD19^+$) were cultured to eliminate cross-contamination of cell subset with other cells, and confirm that the detection of HCV-RNA in purified PBMC subpopulations was caused by active replication rather than by passive adsorption of virions or contamination with serum-associated viral particles.

Purification of cells was performed by microbeads separation (Milteny Biotec, Bergisch Gladbach, Germany) at standard condition; after flow-cytometric analysis by FACScan (Becton Dickinson, Milan, Italy), the purity of sorted cells was provided to be 95% to 97%. Mitogenic proliferation of cultured PBMC and subset populations was evaluated by [^{3}H] thymidine incorporation. Then, HCV-RNA was detected in serum and cells by reverse transcription-nested-polymerase-chain reaction (RT-n-PCR) for the highly conserved 5′ untranslated region of HCV genome.[2] Minus-strand RNA was detected by using a sense primer instead of an antisense primer for RT. To verify the homogeneity of total RNA extraction from different cell subpopulations, a 10-fold sample dilution of purified cells was amplified for a β-actin gene, showing comparable positive results. HCV-RNA was found to be positive in fresh PBMC and resting B lymphocytes of 3 patients, whereas $CD4^+$ and $CD8^+$ resting cells were HCV-RNA positive in only 1 of these 3 patients. HCV genomic sequences were undetectable in fresh total PBMC and subpopulations of 1 other patient. The presence of HCV-RNA was then searched for in total PBMC, $CD4^+$, and $CD8^+$ cells at days 7 and 14, and in B lymphocytes at days 7 and 9 of mitogenic stimulation. In 3 of 4 patients, B lymphocytes were HCV-RNA positive at day 7 of stimulation whereas HCV-RNA was found to be negative in cultured $CD4^+$ and $CD8^+$ subsets of all the patients (Fig 1).

To further confirm our data, the negative-strand RNA, which is a replicative intermediate of HCV, was searched for in serum, total PBMC, and in the subset of cells that were previously found to be plus-strand RNA positive. All serum samples were found to be minus-strand RNA negative. The antigenomic-RNA was detected after n-PCR in the PBMC and B-lymphocyte subpopulation of 1 patient, both in unstimulated cells and after 1 week of mitogenic stimulation (Fig 2).

Finally, HCV genomic sequences were sought in culture supernatants, and were undetectable in culture media of all the cultured cells, suggesting that viral particles are not being released. We found constantly the presence of HCV-RNA in resting and stimulated (with pokeweed mitogen at final concentration of 2.5 μg/mL) B lymphocytes, whereas $CD4^+$ and $CD8^+$ subpopulations were invariably negative after stimulation. In the present study, the minus-strand RNA, which is described to represent an intermediate replicative form of HCV,[3] was detected in fresh and cultured PBMC and B lymphocytes of 1 patient, whereas antigenomic sequences were not detected in the B lymphocyte subset of 2 other patients. It is possible that these patients had a small quantity of minus-strand RNA, which is known to be in a lower concentration compared with positive-strand RNA,[4] or HCV was present but dormant, also after stimulation of cells. Recently, Pileri et al[5] showed that the HCV E2 envelope protein binds to CD81, a tetraspanning expressed in various cells including hepatocytes and B lymphocytes. In particular, this study showed that HCV binds to human B-cell lines. In agreement with these data, we invariably found HCV-RNA in quiescent as stimulated B lymphocytes. In conclusion, we showed the presence of HCV plus and minus-strand RNA in cultured total PBMC and B cells, and these findings strongly suggest that PBMC and, in particular B lymphocyte subsets, may represent extrahepatic sites of HCV replication. Further studies on in vivo and in vitro replication of HCV in PBMC subsets could help to elucidate the viral life cycle in PBMC.

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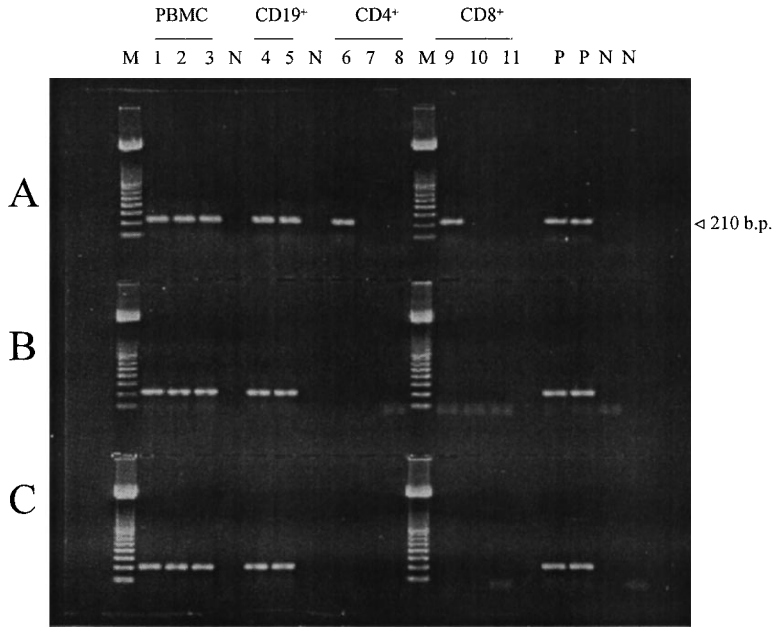

Fig 1. Ethidium bromide–stained agarose gel of HCV-amplified products after nested-PCR of PBMC and PBMC subsets of 3 patients (A, B, C, respectively) before and after mitogenic stimulation of cells. Lanes 1 through 3, PBMC at day 0, 7, 14 of stimulation; lanes 4 and 5, B lymphocytes at day 0, 7; lanes 6 through 8 and lanes 9 through 11, CD4+ and CD8+ cells at day 0, 7, 14 of stimulation, respectively. Day 0 corresponds to unstimulated cells. N, negative controls (serum samples from healthy blood donors); P, positive controls (serum samples with a known amount of HCV-RNA); M, DNA size markers.

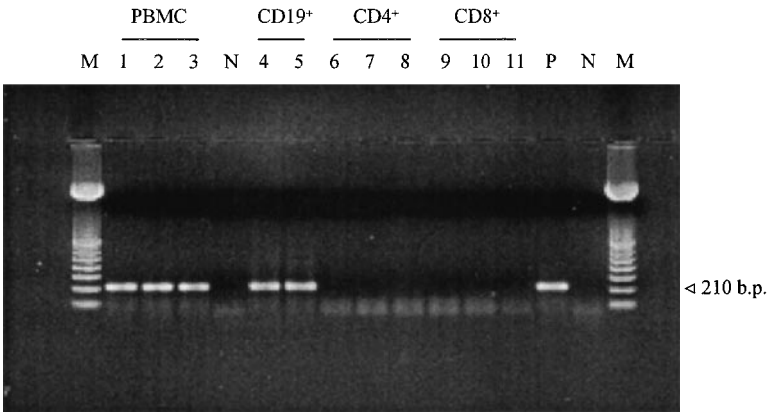

Fig 2. Detection of HCV-RNA minus strand after ethidium bromide–stained agarose gel of HCV-amplified products of 1 patient with chronic hepatitis, before and after stimulation of cells. Lanes 1 through 3, total PBMC at day 0, 7, 14; lanes 4 and 5, B lymphocytes at day 0, 7; lanes 6 through 8 and lanes 9 through 11, CD4+ and CD8+ cells at day 0, 7, 14 of stimulation, respectively. Day 0 corresponds to unstimulated cells. M, DNA size markers; N, negative control; P, positive control.

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Genetic Heterogeneity of Congenital Dyserythropoietic Anemia Type I

To the Editor:

Congenital dyserythropoietic anemia type I (CDA type I) is an uncommon disorder of unknown etiology characterized by a variable degree of anemia, macrocytosis, internuclear chromatin bridges between incompletely separated erythroblasts, a striking ultrastructural abnormality (spongy or 'Swiss-cheese' appearance) of erythroblast heterochromatin, grossly ineffective erythropoiesis, and autosomal-