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Expansion of CD3+CD56+ cytotoxic cells from patients with chronic lymphocytic leukemia: *in vitro* efficacy

Cytokine-induced killer cells were expanded from 12 patients with chronic lymphatic leukemia. In these cultures, T-cells increased significantly from less than 10% to 56.3± 29.4% after 14 days. Similarly, the percentage of cells expressing the natural killer-cell marker CD56 increased significantly to 31.8±26.3%.

Sir,

Cytokine-induced killer (CIK) cell cultures were generated from 12 patients with chronic lymphatic leukemia (CLL) and assayed for their expression of various cell surface markers by flow cytometry. On day 0 all patients had at least 90% CD19 positive lymphocytes in their blood. After two weeks of culture CD19 positive cells had decreased significantly to 33.3±30.5% with the range being 1.5 and 78.6% (p = 0.02; Figure 1). In contrast, fewer than 10% of the lymphocytes were $CD3^+$ on day 0 of culture. Expression of CD3 increased to 56.3±29.4% after two weeks of culture (p = 0.03). Similarly, CD8 positive cells increased to 53.8±31.4% after two weeks (p = 0.08). The percentage of CD56 positive cells increased significantly to 11.0±11.1% after one week of culture and to 31.8±26.3% after two weeks (p = 0.01; Figure 1). CD56 positive cells co-expressed CD3. Next, we tested the cytotoxic activity of CIK cells using a ^{51}Cr release assay. Fourteen-day old CIK cells were tested using autologous or allogeneic leukemia cells as targets. In the autologous setting CIK cells were unable to lyse leukemia cells. However, CIK cell lysis could be increased by addition of anti-CD3 monoclonal antibody. Addition of anti-CD32 antibody did not abolish this effect. In contrast, addition of anti-CD19 antibody did not produce an increase in cytotoxicity. In the allogeneic setting CIK cells showed a weak cytotoxic effect on leukemia cells. Again, this effect could be increased by addition of anti-CD3 antibody. This effect was not abolished by addition of anti-CD32 (Figure 2).

CLL cells are resistant to T-lymphocytes.

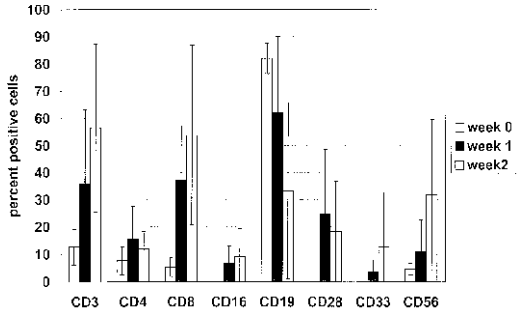

Figure 1. Flow cytometric analysis of various cell surface markers on CIK cell cultures derived from CLL patients. Expression was analyzed by flow cytometry as described elsewhere.[3] Data are shown as the mean from twelve separate experiments. Please note that data on day 0 are derived from three patients only and that CD16, CD28 and CD33 were not determined on day 0.

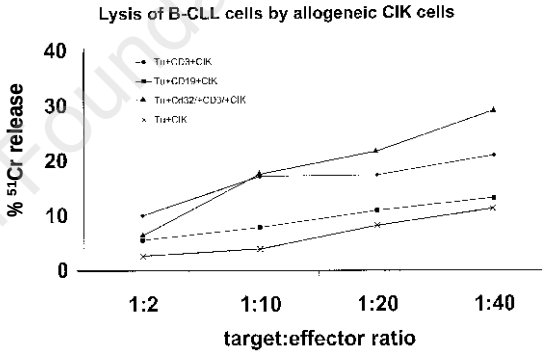

Figure 2. Cytotoxic activity of CIK cell cultures derived from CLL patients. CIK cells were generated from CLL patients as described before.[4-7] After 14 days of culture, cells were assayed in a ^{51}Cr release cytotoxic assay with or without addition of anti-CD3 monoclonal antibody at various effector to target cell ratios in an allogeneic setting. In the control experiment anti-CD19 antibody was used instead of anti-CD3. In addition, anti-CD32 was added to anti-CD3 in some experiments. Results shown represent data from two separate experiments. Data are shown as means.

Recently, we showed that CIK cells can be directed to leukemia and lymphoma cells via reverse antibody-dependent cellular cytotoxicity.[1] There was an increase in sensitivity to CIK-mediated lysis of various lymphoma and leukemia cell lines by preincubation of the targets with a monoclonal antibody against CD3. This increase could be partially blocked by preincubation with anti-CD16 (Fc receptor III) and anti-CD32 (Fc receptor II) antibodies. These data suggest that the increase in cytotoxic activity is due to Fc receptor-mediated antibody binding. Cytotoxic activity could be further increased by addition of an anti-CD28 antibody to anti-CD3. In accordance, we show here that the addition of an anti-CD3 antibody leads to an increase in cytotoxic activity of CIK cells against CLL cells. CIK cells are effective against allogeneic leukemia cells.[2] However, there was only a minor effect against autologous leukemia cells. We speculate that the reason for this resistance lies in the lack of co-stimulatory signals on the cell surface of CLL cells. Further studies will concentrate on activating CIK cells on autologous CLL cells.

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Key words

NK cells, T cells, CIK cells, CLL.K

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Non-ALC peripheral T-cell lymphomas in children: report on two cases and a review of the literature

Peripheral T-cells lymphomas (PTCLs) in children are usually good prognosis Ki1+ ALCL; other PTCLs have the same poor prognosis as in adults.[1,2] We report the cases of two children with PTCL, whose disease had an aggressive clinical course. There are only scanty reports dealing with optimal therapy for this rare disease. Considering the bad prognosis shared by adults and children, a common study is recommended.

Case reports

The two clinical histories are summarized in Table 1 and the methods used for histopathologic and molecular biology studies are illustrated in Table 2.

At light microscopy (3rd biopsy, case #1, 3rd and 4th biopsies, case #2) both cases showed effacement of lymph node architecture with increased vascularity and branching endothelial venules. The neoplastic cells were small-medium sized with polymorphic nuclei, small nucleoli and scanty, pale gray cytoplasm. There were some large, basophilic blast cells and a moderate number of mitotic figures. The neoplastic cells were obscured by epithelioid histiocytes, polyclonal plasma cells, eosinophils and hyperplastic clusters of follicular dendritic cells (Figure 1) and a polymorphous cellular infiltrate of plasmacells, eosinophils, histiocytes and numerous immunoblasts. The T-cell origin of the NHL was derived from the pattern of immunoreactivity (CD 3^+, $CD4^+$, $CD7^+$, $CD8^-$, $CD19^-$, $CD20^-$, $CD22^-$, $CD30^-$, $CD79a^-$, TdT^-). The initial biopsy of case #1 was viewed by RG: the lymphoid tissue was composed of a mixed population with immunoblasts scattered among small lymphocytes; small areas of necrosis were present without multinucleate giant cells. In the second biopsy of case #1, the nodal architecture was severe-