not contain associated adhesin domains in contrast to HrgpA) was able to activate human neutrophils through the PAR-2 receptor. Thrombin does not activate PAR-2, and thus, combining this with other data on the specificity of the gingipains, we cannot entirely agree with the contention of McRedmond and Fitzgerald that the gingipains appear to have a thrombinlike specificity.

McRedmond and Fitzgerald now make the case for similarities between the action of gingipains on the PARs and that seen with the overall streptokinase-plasminogen-antistreptokinase complex. In particular, the authors focus upon the similar function of the associated adhesins of HRgpA in aiding the cleavage of the PARs, much as the streptokinase-plasminogen complex is guided to the platelet surface by the binding of the antistreptokinase antibodies to the platelet Fc receptor, as described in their work. Recent work in our laboratory has shown that HRgpA and RgpB in fact have some differences in their specificity for peptide substrates that may relate to small differences in their active site architecture induced by a limited number of point mutations (N. Alwy et al, manuscript in preparation). We cannot rule out that the adhesins of HRgpA play some role in enhancing functions such as cleavage of coagulation factors and PARs, but it would certainly appear that we can no longer be sure that it is only these adhesins that are modifying the action of the larger protein. Certainly, it must be noted that RgpB can activate the PARs directly and therefore the adhesin domains are not as vital in this process as in the streptokinase scenario.

The letter by McRedmond and Fitzgerald now amplifies upon the role of streptokinase-induced cellular activation in bacterial pathogenesis. The scenario posed by them is certainly a fascinating one and, together with our recently published work on the activation of PARs on epithelial cells by the gingipains and the resulting induction of interleukin-6 secretion, the data obtained with streptokinase certainly aids in establishing our proposal that PAR-mediated activation of mammalian cells by bacterial proteins is an intriguing new mechanism to be investigated in bacterial pathogenesis. The importance of PAR-mediated platelet activation by bacterial proteins also needs to be established further, which will provide further intriguing glimpses into the possibility that cardiovascular diseases might, in some cases, have an underlying chronic bacterial disease as the cause.

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References


To the editor:

Terminal plasmocytoid differentiation of malignant B cells induced by autotumor-reactive CD4+ T cells in one case of splenic marginal zone B-cell lymphoma

In lymphoid organs invaded by malignant B-cell lymphomas, the development of reactive CD4+ tumor-infiltrating T cells (TIL-T) at the contact of tumor cells is now firmly established. It has been reported that lymphoma B cells are capable of proliferating in response to various recombinant signals usually provided by CD4+ T cells, such as interleukin-4 (IL-4), or CD40 ligand. However, the exact nature of the functional relationships between CD4+ TIL-T and autologous non-Hodgkin lymphoma (B-NHL) cells remains largely unknown mainly because this question has not yet been extensively investigated in autologous CD4+ T/malignant B-cell coculture systems in vitro. We report here evidence that CD4+ TIL-T have the potential to drive autologous lymphoma B cells toward a terminal differentiated state, in one case of splenic low-grade, marginal zone B-cell lymphoma.

In May 1998, a 67-year-old man presented with a low-grade lymphoma (nonfollicular small-cell lymphoma) with involvement of spleen, blood, periaortic lymph nodes, liver, and bone marrow. The patient underwent splenectomy in July 1998. Histologic, cytologic, and immunophenotypic features were compatible with splenic marginal-zone B-cell lymphoma. All malignant B cells were surface IgM+, kappa+, CD19+, CD24+, CD40+, and were negative for IgD, CD23, and CD5. Less than 1% of malignant cells were CD38+ or CD138+. The percentage of CD3+ TIL-T was 9% with 5% CD4+ and 4% CD8+.

A population containing both CD4+ T cells and malignant B cells was negatively selected from total spleen cells by depleting CD8+ T cells, residual NK cells, monocytes, and normal B cells by one round of immunomagnetic bead depletion. Purity was assessed by flow cytometry and CD4+ T/malignant B-cell preparations usually contained 8% to 10% CD4+ T cells and 88% to 90% CD19+ kappa+ B cells. Residual cells not stained by CD3, CD4, CD19, or kappa antibodies were always less than 1%. Cocultures were then performed in the presence of recombinant IL-2 (rIL-2) at 10 IU/mL. Control cultures consisted of purified malignant B cells cultured with rIL-2 but without the presence of CD4+ T cells.

A representative experiment of CD4+ T/malignant B-cell cocultures is given in Figure 1. After 7, 14, and 21 days of coculture, cells were harvested, triple stained with anti-CD4-PE-Cy5, kappa-PE, and CD138-FITC antibodies (Abs) and analyzed by flow cytometry. CD138 (Syndecan-1) is a transmembrane heparan sulfate proteoglycan expressed in Ig-producing, normal and malignant mature plasma cells. At analysis, according to a multicolor gating/painting strategy, CD138+ cells were gated and colored in orange, and CD138- cells were colored in gray. At the initiation of the coculture (day 0), the spontaneous formation of conjugates between T cells and malignant B cells was negligible. At the contact of malignant B cells, autotumor-reactive CD4+ T cells became activated, expanded, and formed stable conjugates with malignant B cells. Between day 7 and day 21 of the coculture, the percentage of T/B-cell conjugates gradually increased (5% at
day 7, 36% at day 14, and 34% at day 21), and this was accompanied by a progressive increase in the number of free T cells (from 6% at day 0 to 51% at day 21). CD138⁺ cells progressively accumulated in the CD4⁺ T/B-cell coculture and reached a maximum value of 18% of the total number of cells at day 14 (1%, 4%, 18%, 13%, at days 0, 7, 14, 21, respectively). CD138⁺ cells were almost exclusively confined within CD4⁺ T/B-cell conjugates (Figure 1), suggesting that the acquisition of this ag by malignant B cells was dependent on a close association with autotumor-reactive CD4⁺ T cells. To formally show that the CD138⁺ cells were truly plasma B cells, these cells were positively purified by an immunomagnetic method at day 14 of the coculture and phenotyped (Figure 2). This method allowed us to obtain a very pure population of CD138⁺ cells (98%). As expected, CD3, CD19, CD20, and CD24 were low or absent; the expression of CD40 was intermediate, and kappa light chain was expressed at a low level (mean value: 198 units of fluorescence) in comparison to fresh malignant B cells (mean value: 1299). A high proportion (85%) of cells strongly expressed CD38, a relevant marker to plasma cells. These cells exhibited a morphology of plasma cells and contained large amounts of intracytoplasmic kappa chain as estimated on cytospin slides stained with fluorochrome-conjugated anti-kappa Abs. To ensure that these CD138⁺ plasma cells belonged to the neoplastic clone, IgH gene rearrangements of purified CD138⁺ cells were investigated by a PCR-based method using Fr3 region primers and compared with IgH gene rearrangements of purified fresh malignant B cells. Both cell populations showed the same rearrangement bands, stressing that the CD138⁺ cells were B cells and clonally related to the original lymphoma cells (Figure 3). Cell-division tracking using PKH26 dye labeling indicated that more than 95% of the malignant B cells remained in a nonproliferative state throughout the coculture, formally excluding that the accumulation of CD138⁺ cells during the CD4⁺ T/malignant B-cell coculture might arise from the rapid proliferation of a minor component of CD138⁺ cells present at the initiation of the coculture. Finally, in control experiments, purified malignant B cells cultured alone in the sole presence of rIL-2 retained a phenotypic profile identical to...
fresh malignant B cells without any down-regulation of CD19, CD20, CD40, and surface Ig, or up-regulation of CD38 and CD138. Thus, malignant B cells did not show any tendency to spontaneous or rIL-2–induced differentiation in the absence of autologous CD4+ T cells.6-10

In the literature, there are now some convincing observations supporting the notion that B-lymphoma cells could be released from their apparent maturation block by external signals. Cerutti et al6 have described that in Burkitt lymphoma, a monoclonal B-cell line was induced to progress throughout a phenotypic differentiation program that approximated the stages of early centroblast, centroblast, centocyte, and memory B cells, after engagement of CD40 by CD40 ligand and exposure to IL-4 and IL-10. In the presence of IL-6, these cells were driven to terminally differentiated plasma cells. Recently, a second experimental model used a follicular dendritic cell line, HK, and a lymphoma line, L3055, that resembles centroblasts. L3055 cells proliferated continuously in the presence of HK cells, whereas they differentiated into a population with the phenotype of centocytes after stimulation with CD40 ligand and IL-4.7 In follicular lymphomas, it was formerly reported that allogeneic T cells induced fresh malignant B cells to secrete large amounts of IgG, and this was accompanied by loss of the surface Ig and development of abundant intracytoplasmic IgG.8

In 1995, Kramer et al9 reported a patient with follicular lymphoma who showed in vivo differentiation of malignant cells after autologous bone marrow transplantation and treatment with IL-3. The patient developed a plasmocytosis in blood and bone marrow accompanied by a paraprotein corresponding to the malignant clone and high IL-6–serum concentration. It was proven that the plasma cells were clonally related to the original lymphoma cells. The patient recovered spontaneously and had long-lasting remission, suggesting that terminal differentiation of malignant B cells was beneficial to the patient. More recently, Dogan et al10 described that follicular lymphomas contain a clonally linked but phenotypically distinct neoplastic B-cell population in the interfollicular zone. It was demonstrated that the immunophenotype of the neoplastic interfollicular cells was similar to that of a subpopulation of postfolicular B cells observed outside the folliciles in normal lymphoid tissue, with down-regulated expression of follicle-center activation markers such as CD10, CD38, CD80, CD86, and CD95. These cells are thought to be malignant memory B cells that have differentiated from the malignant follicle-center B cells. Thus, differentiation towards a cell with more mature phenotype should be a possible occurrence in low-grade B-cell lymphoma.

Marginal-zone lymphomas tend to have an indolent clinical course.9 In the current case, the patient has remained stable without any treatment after undergoing splenectomy 35 months ago. The results presented here would provide an explanation for this: the tumor cells may have received signals from autotumor-reactive CD4+ T cells leading to a continuous process of differentiation that may be beneficial to the patient. As demonstrated in the current study, in-depth analysis of CD4+ T/malignant B-cell conjugates in CD4+ T/malignant B-cell cocultures should form the basis for valid evaluation of the effects of autotumor-reactive T cells on malignant B cells: induction of differentiation but also growth regulation/apoptosis. These immunologic criteria would provide a strong rationale to engage individual cellular immunotherapy with autologous CD4+ T cells in B-cell NHL.

References


To the editor:

Cytomegalovirus infection in cancer patients receiving granulocyte transfusions

Cytomegalovirus (CMV) infection still has a high mortality rate especially if one develops pneumonia.1,2 Although studies have shown the relationship between leukocytes in blood products and the risk of transfusion-acquired infection, we suggest that the risk of granulocyte transfusion–acquired CMV infection in patients with cancer appears to be low. Prevention of CMV infection requires screening the donors for CMV antibody or leukoreduction by filtration, which obviously cannot be done for granulocyte transfusion.3,4 Furthermore, the prevalence of CMV antibody in our donor population is high, and using donors that are CMV seronegative would therefore remove 70% to 80% of available granulocyte donors.

We retrospectively reviewed 100 cases of cancer patients who received CMV–unscreened-granulocyte transfusions from January 1995 through April 2001 with the following diagnosis: acute myelogenous leukemia, 41; chronic lymphocytic leukemia, 10; chronic myelogenous leukemia, 8; multiple myeloma, 1; refractory anemia with excess blast, 1; acute promyelocytic leukemia, 1;
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