We investigated the clonal relationship of malignant cells in a patient affected with both chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). CLL cells and malignant plasma cells synthesized IgG κ and IgA κ molecules, respectively; these monoclonal Ig shared idiotypic determinants, providing evidence that a single clonal disease occurred in this patient. Furthermore, when leukemic CLL cells were driven to differentiate in vitro to immunoblasts and plasma cells, a switch from IgG to IgA occurred in a significant percentage of cells that were double producers. These data suggest that, in some circumstances, CLL leukemic B cells may reach a more mature state, leading to the occurrence of clinical MM.

The ascending limb of the first peak was concentrated by vacuum dialysis; it contained mainly the monoclonal IgA κ and a few contaminating proteins as assessed by double immunodiffusion using antisera to IgA, IgG, IgM, and a polyvalent rabbit antiserum to human serum. An anti-idiotypic serum was prepared in rabbits by repeated footpad or subcutaneous (SC) immunizations with 350 μg of the purified IgA in Freund's adjuvant. Immune serum was absorbed with polyclonal IgG, IgM, and IgA. In double diffusion experiments, the absorbed serum reacted only with the patient's IgA κ. By indirect immunofluorescence, this serum was negative on pokeweed mitogen (PWM)-stimulated PBLs, which contained a fair percentage of IgA, IgG, and IgM plasma cells.

**Immunofluorescent procedures.** Surface and cytoplasmic Ig were characterized by direct immunofluorescence using rhodamine or fluorescein-conjugated Fab′2 fragments of rabbit IgG specific for μ, γ, δ, κ, λ and κ λ chains as described elsewhere. Determination of IgG subclass of surface and cytoplasmic IgG synthesized by the patient's leukemic cells was performed by using monoclonal antibodies to the various IgG subclasses (a kind gift of Dr. J.D. Capra) and a second layer of goat antibodies to mouse Ig. Double-labeling for surface and cytoplasmic IgG were performed by first staining living cells with rhodamine reagents; after three washings, cells were smeared on slides, fixed in 95% ethanol, and thereafter were restained for cytoplasmic IgG with fluoresceinated antibodies. Reactivity of the anti-idiotypic serum was studied by indirect immunofluorescence, using as a second layer Fab′2 fragments of goat IgG antibodies to rabbit IgG that had no cross-reactivity with human Ig by direct immunofluorescence.

**RESULTS**

Membrane markers of leukemic CLL cells and malignant plasma cells. Nearly 100% of blood lymphocytes featured a dim surface staining with anti-γ and -κ sera; no positive cells were seen with anti-μ, -α, -δ, or -λ antibodies. Intracytoplasmic staining of CLL cells showed numerous crystalline inclusions brightly reactive with anti-γ and -κ antibodies. These IgG molecules belonged to the γ1 subclass. Besides

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small lymphocytes, the bone marrow was infiltrated with plasma cells containing IgA\textalpha{} molecules. No plasma cells (or IgA-positive cells) were detected among PBLs.

**Induction of differentiation of leukemic CLL cells.** In order to induce differentiation of leukemic lymphocytes, the patient's PBLs were cultured in the presence of different mitogens and of allogeneic T cells. The Ig phenotype of the stimulated cells was determined after various times of culture. Table 1 indicates results of representative experiments. No modification was observed in unstimulated cultures. In the presence of mitogens and/or allogeneic T cells or T cell conditioned medium, two main changes were observed. At day 8, a few blast cells bearing surface \textalpha{} chains were seen; double-labeling experiments for surface and cytoplasmic Ig showed that some of these surface \textalpha{}-positive cells exhibited simultaneously characteristic crystalline y inclusions. At day 12 of culture, cytoplasmic immunofluorescence revealed that 3% to 10% of the cells contained \textalpha{} chains (Table 1); some had the morphological aspect of plasma cells. Some of these cells also contained y heavy chains, as shown by simultaneous staining with rhodamine anti-\textalpha{} and fluorescein anti-y antibodies. In the latter cells, the characteristic aspect of the crystalline inclusions was often modified because of a decrease in the number of crystals.

**Idiotype studies.** An anti-idiotype rabbit serum was raised against the patient's serum monoclonal IgA. After suitable absorptions, its specificity was confirmed by lack of staining of polyclonal plasma cells. This antisera labeled by indirect fluorescence the cytoplasmic y as crystalline-like crystals inclusions of leukemic CLL B cells. The staining was abolished by further absorption of the antiserum with patient's IgA\textalpha{} but not by unrelated IgA\textalpha{}.

**DISCUSSION**

The clinical and hematological diagnosis of CLL is not questionable in patient L.H.E. The immunologic characterization of the leukemic CLL cells revealed a homogeneous proliferation of lymphocytes featuring surface and cytoplasmic IgG1\textalpha{} molecules. For ten years, the patient had a marked blood and bone marrow lymphocytosis when MM occurred: lytic bone lesions developed, with progressive infiltration of the bone marrow by malignant plasma cells that secreted IgA\textalpha{} molecules. The association in one patient of CLL and MM is quite rare, since less than 40 cases have been reported.\(^1\) In most cases, as in the present one, CLL has been diagnosed one to 15 years before MM. The clinical picture is usually a composite of typical features of both diseases; however, the incidence of extrasosseous plasma cell tumors may be outlined. The class distribution of serum or urinary monoclonal components of MM supervening on CLL appears rather unusual, since IgA cases predominate over IgG, light chains, IgM, IgD, or nonsecreting myelomas. To account for this rare association, two main hypotheses are currently offered: the malignant B CLL clone might further mature to give rise to a proliferation of plasma cells; alternatively, the two diseases could represent distinct clonal proliferations. Immunologic studies performed so far favor the latter hypothesis. In six of 12 cases studied,\(^1,3,7\) CLL cells and plasma cells synthesized Ig molecules with different light chain types, a widely accepted although circumstantial evidence for distinct clonal origin. Moreover, in three cases in which surface Ig of CLL cells and cytoplasm Ig of plasma cells had the same light chain type, they did not share idiotypic determinants.\(^13\) It should be pointed out, however, that the finding of two clones synthesizing idiotypically different molecules (or even Ig molecules with different light chain types) does not definitely eliminate a clonal disease affecting a precursor cell. Indeed, somatic mutations may modify idiotopes on one cell line and, on the other hand, the occurrence of a common V\textsubscript{H} gene rearrangement with different V\textsubscript{\textkappa{}} gene rearrangements in different subclones has been suspected in a recent study.\(^1\) In contrast, in the present report, CLL cells and malignant plasma cells synthesized IgG and IgA molecules, respectively, that shared common idiotypic determinants. Indeed, a rabbit anti-idiotypic antiserum to the serum monoclonal IgA stained the cytoplasmic IgG crystalline-like inclusions characteristic of the patient's CLL cells.

Furthermore, we obtained in vitro evidence that CLL cells were the precursors of IgA plasma cells. To explore further the clonal relationship between the two diseases in our patient, we investigated whether leukemic CLL cells could be induced to differentiate in vitro.\(^4,11\) According to the stimulus used, 3% to 10% \textalpha{}-synthesizing immunoblasts or plasma cells were observed in late cultures (at day 12) of

<table>
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<th>Culture Conditions and Day of Culture</th>
<th>Medium Alone</th>
<th>PHA</th>
<th>SAC + PHA + Allogeneic T Cells (50%)</th>
<th>SAC + T Cells Supernatant Added at Day 3 of Culture</th>
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<td>0</td>
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<td>12</td>
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<td>slg, surface immunoglobulin; clg, intracytoplasmic immunoglobulin; PHA, phytohemagglutinin; SAC, S. aureus; ND, not done.</td>
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PBLs that initially contained over 95% monoclonal γκ bearing cells. That these α cells did not originate from precursors or residual B cells could be ascertained by double-staining experiments that disclosed cells having both cytoplasmic α and γ chains; the latter cells displayed the crystalline inclusions characteristic of the patient’s CLL cells. Finally, earlier in culture (at day 8), cells with surface ακ molecules and cytoplasmic γκ inclusions were observed. Altogether, these findings offer strong evidence that the leukemic CLL cells could be induced to differentiate in vitro with a switch from IgG to IgA synthesis.

The in vitro switch we observed was triggered by T cells (or T cell factors) as it probably occurs in vivo for normal B cells. This finding raises the important issue of whether leukemic clones may be sometimes susceptible to regulation by physiologic T mediators or cognate interaction with T cells. To what extent such an event occurs in vivo in our patient is presently unknown. On the other hand, it should be emphasized that there are many exceptions to the conventional view that leukemic CLL cells are frozen at a given step of maturation. Apart from the possible maturation and switch to plasma cells as was shown in this patient, CLL cells more often transform into large blastic cells (the so-called Richter’s syndrome) having usually (but not always) the same clonal origin.12.13

The occurrence of in vitro heavy chain switch is remarkable and has been seldom noted in human lymphoid malignancies. Rudders14 reported a patient with CLL having two leukemic clones featured by idiotypically related γκ and ακ molecules; in this patient, a small percentage of cells synthesized both molecules. In vitro experiments in two other CLL patients demonstrated that stimulated leukemic cells were able to mature in vitro, with a switch from IgM to IgG synthesis.9,15 We have no data about the molecular events implied in the switch phenomenon we observed. However, the occurrence of cells synthesizing both IgG and IgA and of IgA plasma cells suggests two possibilities that are not mutually exclusive. First, the Cγ1 and Cα1 genes could be cotranscribed and expressed by an RNA processing mechanism as occurs in B lymphocytes coexpressing IgM and IgD. A long transcript would be needed, since γ1 and α1 genes are separated by 32 kb. Such a model of a large multi-CH gene transcript has experimental support.16 Alternatively, γ1, Cγ2 gene (and γ5 constant genes) may be deleted, as occurs universally in plasmacytomas and also in murine B cells stimulated by polyclonal activators17; in this case, a long-lived γ chain mRNA could account for our finding of double producers. Whatever the mechanism involved, such a disease association offers a unique opportunity for the study of the heavy class switch in B cells. For instance, our data strongly suggest the existence of a switch from IgG to IgA, whereas most studies of the differentiation of human circulating B cells are rather consistent with the occurrence of direct switches from μ to each of the other isotypes.18

REFERENCES


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Associated chronic lymphocytic leukemia and multiple myeloma: origin from a single clone

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