Brief Definitive Report

IgE SYNTHESIS BY CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Chronic lymphocytic leukemia (CLL) is characterized by the clonal proliferation of B lymphocytes that are "arrested" at the intermediate stage of their differentiation (1). B cells isolated from CLL (B-CLL) display surface Igs (typically μ, sometimes μ and δ, and rarely γ or α) bearing the same idiotype and L chain type (κ or λ, never both). The hallmark of B-CLL cells is the expression of CD5 antigen at their surface. $CD5^+$ B cells constitute a minor subpopulation of normal adult PBMC (1–6%) (2). B-CLL cells are not irreversibly frozen at an immature level of the B cell development; they differentiate into IgM-secreting cells upon stimulation with phorbol esters (3, 4), polyclonal B cell activators (5), and less frequently, under the influence of IFNs (γ, α, or β) (6) or IL-2 (7). However, the ability of B-CLL cells to undergo a γ H chain class switching is a controversial issue (8, 9).

IL-4 is a potent inducer of human IgE synthesis by normal PBMC (10). In the rodent system, IL-4 has been shown to cause the isotype switching to IgE (11). Nevertheless, the IgE precursors cells are not well defined. Earlier studies in the animal model suggested that these cells might belong to a discrete B cell subset (12). Very recently, some authors reported the cloning and the molecular characterization of human IgE-committed B cells (13). Their conclusion supports the previous view that these cells do represent a unique B cell sublineage. In the present paper, we examined the ability of CD5 malignant B cells to differentiate into IgE-secreting cells in response to IL-4. The results indicate that hydrocortisone (HC) is required together with IL-4 to trigger an IgE response by CLL lymphocytes.

Materials and Methods

Cells Culture. 15 untreated CLL patients, classified according to Rai staging (14), were selected for this study. The blood levels of their monoclonal B lymphocytes ranged from 1.2×10^4 to 10^5 cells/mm^3. Normal adult volunteers were used as controls. PBMC were separated by density gradient centrifugation as previously described (10).

PBMC were cultured at a concentration of 10^6/ml in 96-well flat-bottomed plates in HB101 medium (Hana Biologics, Berkeley, CA) supplemented with 5% FCS (Gibco Laboratories, Grand Island, NY). rIL-4, obtained from either Genzyme (Boston, MA) or from Dr. H. Hofstetter (Ciba Geigy, Switzerland), was used at the concentration of 400 U/ml. HC was used at concentrations ranging from 10^{-4} to 10^{-7} M (Sigma Chemical Co., St. Louis, MO)

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and cycloheximide (CHX) (Sigma Chemical Co.) was used at 100 μg/ml. After a 12-d culture, cell viability was ~95%, except in the cultures supplemented with CHX.

Immunofluorescence. Fresh CLL cells were stained for surface Ig L chain (SIg^+) and CD5 and CD23 antigen expression using FITC-$F(ab')_2$ goat anti-human κ or λ (Tago Inc., Burlingame, CA), at a dilution of 1:100, and phycoerythrin-conjugated anti-CD5 (Becton Dickinson & Co., Mountain View, CA), and biotinylated anti-CD23, followed by FITC-avidine at a dilution of 1:50, respectively. The proportion of stained cells (percent) was measured by flow cytometry (FACScan; Becton Dickinson & Co.). For the detection of intracytoplasmic IgE (IC^+ IgE), PBMC from the same CLL patients were cultured in the presence of a mixture of IL-4 (400 U/ml) and HC (10^{-5} M). After 12 d, the cells were washed, cytocentrifuged onto microscope slides, fixed in ice-cold methanol, and stained with anti-IgE mAb (clone 4.15) (a gift from Dr. Saxon, UCLA) at 5 μg/ml, followed by FITC-$F(ab')_2$ goat anti-mouse Ig (Tago Inc.) at the dilution of 1:40. Fluorescent cells were quantified using a fluorescence microscope (E. Leitz, Inc., Rockleigh, NJ) (apochrome; 63× objective).

RIAs. Total IgE, in the various culture supernatants (CSN), was measured by RIA exactly as previously described (15). Two anti-IgE mAbs were used in this assay. Clone 89 (10 μg/ml), produced in our laboratory, was used to coat the wells of microtitre plate and clone 4.15, as ^{125}I-labeled mAb. The sensitivity of the assay was of 0.2 ng/ml. Specific IgE λ and κ were also measured by RIA. For this assay, the plates were coated with anti-IgE mAb (clone 89). After blocking, the CSN were allowed to incubate for 16 h. After washing, wells were incubated overnight with ^{125}I-radiolabeled $F(ab')_2$ goat anti-human κ or λ (Tago Inc.), washed, and counted. IgE λ (present in the CSN of the U266 IgE myeloma cell line) and IgE κ (isolated from the serum of the IgE myeloma patient ADZ and kindly given by Dr. A. L. de Weck, Switzerland) were used as standards and as specificity controls for the respective assay.

Results

CLL Lymphocytes Synthesize IgE when Stimulated with IL-4 and HC. Mononuclear cells from 11 of 15 CLL patients synthesize IgE in response to a mixture of IL-4 and HC (Table I). The level of IgE production ranges from 1.3 to 54 ng/ml and does not correlate with the number of circulating lymphocytes. The other classes of Ig are not induced in the same cultures (data not shown). It is important to note that CLL cells do not produce IgE in response to either IL-4 (up to 10,000 U/ml) or HC used alone. The concentrations of IL-4 (400 U/ml) and of HC (10^{-5} M) used in these experiments have been selected in preliminary dose-response studies. The four nonresponder patients have been tested twice with negative results, whereas all responders have been tested three consecutive times at a 1-mo interval with positive results. Neither clinical stage nor lymphocyte count can differentiate IgE responders from nonresponders. Moreover, the two groups of patients display similar immunological parameters, i.e., same κ/λ distribution, similar range of CD5 expression (80–95% positive cells), and CD23 expression (60–85% positive cells). In contrast, normal PBMC synthesize IgE upon stimulation with IL-4 alone and this response is enhanced by HC (Table II). The following observations indicate that the IgE detected in the CSN of CLL cells is not preformed or of extrinsic origin: (*a*) IgE cannot be detected in the CSN of either unstimulated cells or of cells costimulated with IL-4 and HC and supplemented with cycloheximide, a protein synthesis inhibitor (Table I); and (*b*) the IL-4- and HC-stimulated cultures contain cells with intracytoplasmic IgE (Ic^+ IgE) (Table III).

CLL Lymphocytes Synthesize Monoclonal IgE. As indicated in Table III, the costimulation with IL-4 and HC induces the tumor cell population and most likely not the contaminating normal B cells to synthesize IgE. Indeed, all the secreted IgE molecules

TABLE I
IgE Synthesis by PBMC from CLL Patients

Patients	Rai stage	White blood cells/ lymphocytes	sIg	IgE				
				Medium	IL-4	HC	IL-4 + HC	IL-4 + HC + CHX
		(× 10^3/mm^3)/%				*ng/ml*		
1	0	28/73	κ	0	0	0	2.5	0
2	III	110/97	κ	0	0	0	1.8	0
3	IV	92/98	λ	0	0	0	1.3	0
4	0	24/75	κ	0	0	0	17.5	0
5	I	56/98	κ	0	0	0	1.4	0
6	I	19/61	κ	0	0	0	54.0	0
7	0	27/55	κ	0	0	0	17.0	0
8	I	66/87	κ	0	0	0	5.7	0
9	0	20/84	κ	0	0	0	19.0	0
10	0	15/80	κ	0	0	0	4.3	0
11	II	22/70	κ	0	0	0	52.0	0
12	0	12/70	κ	0	0	0	0	0
13	II	85/83	λ	0	0	0	0	0
14	0	24/72	κ	0	0	0	0	0
15	II	42/84	κ	0	0	0	0	0

Freshly isolated PBMC from CLL patients were stained for surface Ig L chain expression (sIg$^+$) using FITC-F(ab')$_2$ goat anti-human κ or λ. Stained cells were counted using a FACS analyser (Becton Dickinson & Co.). PBMC from the same CLL patients were also cultured in the presence of IL-4 (400 U/ml) and/or HC (10^{-5} M), as indicated in Materials and Methods. After a 12-d culture, CSN were collected and IgE was measured by RIA, as described in Materials and Methods. The data are the mean values of eight replicate cultures. The coefficient of variation was <20%. No IgE was detected in IL-4- and HC-stimulated cultures in the presence of CHX (100 μg/ml).

express the same L chain type as the freshly isolated monoclonal CD5$^+$ B-CLL cells. The RIAs for the detection of IgE κ and IgE λ are highly specific. Up to 250 ng/ml of IgE λ gives background cpm levels (± 500 cpm; total count, 2 × 10^5 cpm) when reacted with ^{125}I-anti-κ antibody, and similarly, IgE κ does not bind to ^{125}I-anti-λ antibody. Moreover, the IL-4-induced IgE produced by normal PBMC is made of a mixture of IgE κ and IgE λ (data not shown).

TABLE II
Influence of HC on the IL-4-induced IgE Synthesis by Normal PBMC

Added to cultures	IgE			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
	ng/ml			
-	0	0.1	0.4	0
IL-4	25	15	5.5	8.6
IL-4 + HC 10^{-4} M	37	37	9.6	13.6
IL-4 + HC 10^{-5} M	48	79	32	48
IL-4 + HC 10^{-6} M	46	90	33	44
IL-4 + HC 10^{-7} M	23	52	35	11

PBMC from normal volunteers were cultured with IL-4 (400 U/ml) and various concentrations of HC (10^{-4} to 10^{-7} M). The data are the mean values of eight replicate cultures.

TABLE III

Monoclonal IgE Production by CLL Lymphocytes Stimulated by IL-4 and HC

Patient	Rai Stage	$CD5^+$	SIg^+ (κ/λ)	ToT IgE	IgE κ	IgE λ	Ic^+ IgE
		%	%	*ng/ml*	*ng/ml*	*ng/ml*	%
7	0	86	95/0	17	16	0	ND
9	0	85	95/0	20	19	0	ND
10	0	90	85/0	2.7	3	0	5
11	II	96	90/0	52	51	0	6
Standards							
IgE λ (250 ng/ml)					0	248	
IgE κ (250 ng/ml)					240	0	

CLL PBMC from four CLL patients were stained for SIg^+ L chain and CD5 expression, as described in Materials and Methods. The same cells were also cultured with IL-4 and HC. After 12 d, cells were fixed and stained for the presence of intracytoplasmic ϵ chain (Ic^+ IgE) using anti-IgE mAb; CSN were tested by RIA for the measurement of total IgE and IgE κ or IgE λ, as detailed in Materials and Methods.

Discussion

This study indicates that B-CLL cells are capable of differentiating into monoclonal IgE-producing cells and that, in contrast to normal PBMC, both IL-4 and HC are required to trigger IgE synthesis. The mechanisms underlying the apparent permissive effect of glucocorticosteroids (GCS) on the response of B-CLLs to IL-4, as well as their enhancing activity of the IL-4-induced IgE synthesis by normal lymphocytes, are currently investigated. The influence of GCS on human IgE synthesis is not well documented, and contradictory reports have been published (16, 17). However, physiological concentrations of GCS were reported to induce Ig production (IgG, IgA, and IgM) by purified normal B cells in the presence of monocytes and T-replacing factor for steroids (TRF-S). TRF-S is a 40-kD protein produced by $CD4^+$ T cells upon incubation with GCS and IL-1 (18). GCS can also induce the differentiation of B-CLLs into IgM-producing cells after activation with *Staphylococcus aureus* Cowan 1 and IL-2, in the absence of T cells but in the presence of monocytes. The latter may be replaced by IL-1 and IL-6 (19). These data suggest that GCS act by modulating monocyte–B cell interactions. A similar mechanism might account for their potentiating or "permissive" effect on IgE synthesis by normal PBMC and B-CLLs, respectively. Indeed, it was recently shown that the IL-4-induced synthesis by normal PBMC is IL-6 dependent (20), and that B-CLL cells constitutively produce IL-6 (21).

Since the patients included in this study have been tested more than once with the same results, they may be grouped into IgE responders and nonresponders. The two groups do not differ by clinical or hematological parameters. For example, PBMC from all the CLL patients contain >80% $CD5^+$ B cells and from 60 to 85% $CD23^+$ B cells. Moreover, none of the patients have received prior chemotherapy, including GCS. It is therefore anticipated that analysis of the phenotypic and functional characteristics of the B-CLLs from these two groups of patients will generate basic information regarding IgE precursor B cells and/or the cellular interactions leading to their differentiation into IgE-secreting cells. As such, the present study may be viewed

as a new model for the analysis of the molecular and cellular mechanisms regulating IgE synthesis. Indeed, our data strongly suggest that IL-4 and/or HC causes B-CLLs to switch to IgE. This hypothesis is supported by the recent demonstration by Lebman and Coffman (11) that IL-4 induces ϵ H chain switching in Th2-stimulated clonal murine B cell cultures. Finally, the utilization of this novel system to study IgE regulation by monoclonal human B cells presents major advantages over the use of normal PBMC in that it provides access to IgE precursor cells (*a*) in large quantity; (*b*) in a homogeneous stage of maturation; and (*c*) in a highly purified form.

Summary

The present results indicate that B cells isolated from chronic lymphocytic leukemia (B-CLL) from 11 of 14 patients are capable of specifically producing IgE upon costimulation with IL-4 and hydrocortisone (HC). IgE is detected by intracytoplasmic fluorescence staining and by RIA. Clinical, hematological, and immunological parameters (including Rai stage, WBC, Lc, sIg κ/λ, CD5, and CD23 expression) cannot distinguish the IgE responder from the nonresponder patients. IL-4 alone is a potent inducer of human IgE synthesis by normal PBMC and we show here that its effect is strikingly enhanced by HC. The IgE produced by B-CLLs are monoclonal since they display the same L chain type as the freshly isolated $CD5^+$ B-CLLs. We, therefore, conclude that the combination of IL-4 and HC can abrogate the maturation arrest of $CD5^+$ B-CLLs by inducing their differentiation into IgE-producing cells. The present data provide a unique model to study the isotype switching to IgE and the regulation of human IgE synthesis by monoclonal human B cells.

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