

Vaccination against B-cell Chronic Lymphocytic Leukemia with Trioma Cells: Preclinical Evaluation¹

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ABSTRACT

Purpose: Trioma cells are lymphoma cells that have been fused to a hybridoma and have thereby been modified to express an immunoglobulin directed against surface receptors of antigen-presenting cells. Trioma cells that potentially include all lymphoma-derived antigens will be targeted to professional antigen-presenting cells *in vivo*. This allows uptake, processing, and presentation of tumor-derived antigens to T lymphocytes. In a mouse model, vaccination with trioma cells conferred long-lasting, T cell-dependent tumor immunity and was even able to eradicate established lymphomas. Here, we investigated whether this potent approach is effective in the human system.

Experimental design: Malignant cells from 11 patients with B cell chronic-lymphocytic leukemia (B-CLL) were fused to an anti-Fc receptor hybridoma. The resulting trioma cells were extensively characterized with respect to their clonal origin. The induction of autologous tumor-specific T lymphocytes in the presence of trioma and antigen-presenting cells was examined *in vitro* by determining cytokine secretion in coculture assays.

Results: In seven cases, trioma cells could successfully be generated from B-CLL cells. Stimulation of autologous lymphocytes with trioma cells induced a leukemia-specific

T-cell response. Immunostimulatory trioma cells were also obtained from two patients with solid B-cell lymphoma.

Conclusions: Trioma-mediated immunization may be a promising adjuvant treatment of human malignancies of the B-cell lineage, particularly of B-CLL, which has still a very poor prognosis. Our *in vitro* results pave the way for clinical application.

INTRODUCTION

Advanced stage low-grade lymphomas, such as CLL³ (1), still have a poor prognosis. Therefore, innovative therapies are urgently required. In this regard, immunotherapeutic approaches have attracted much interest. Although the Ig Id expressed by B-cell lymphomas is a unique cancer antigen, and lymphoma cells are able to present peptides to T lymphocytes (1, 2), malignant cells can evade the immune attack. This can be explained partly by their inability to deliver costimulatory signals that are required for efficient T-cell activation (3, 4). We have established a new approach to overcome the inefficient induction of immunity by malignant B cells (5). It is based on redirecting tumor cell-associated antigens to endocytosing surface receptors of professional APC. The APCs internalize and process tumor-derived antigens, present immunogenic peptides to T lymphocytes, and deliver the requisite costimulatory signals. It was shown previously that efficient immunity can be induced by targeting antigens to internalizing and activating FcγR that are expressed on APC (6–10). In our approach, lymphoma cells are fused to xenogeneic hybridoma cells that express an Ab directed against FcγR. The resulting trioma cells potentially harbor all antigens derived from the parental lymphoma, and, in addition, they express the FcR-specific Ig. The rationale of the approach is outlined in Fig. 1. Injection of trioma cells into mice induced a long-lasting tumor immunity that was strictly dependent on CD4⁺ and CD8⁺ T cells (5). Even established lymphomas were efficiently eradicated (11). Thus far, the trioma approach is the most potent regimen for immunotherapy of lymphoma that has been described in an animal model.

As shown in the mouse model, an Id-specific immune response can indeed be elicited by trioma vaccination; however, anti-Id immunity plays only a minor role in tumor protection *in vivo* (12). Instead, the efficacy of the approach is attributable to: (a) a polyvalent vaccination against multiple tumor antigens that

Received 1/3/03; revised 5/6/03; accepted 5/6/03.

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¹Supported by HGF-Strategiefonds "Infektionsabwehr und Krebsprävention."

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³ The abbreviations used are: CLL, chronic lymphocytic leukemia; B-CLL, B-cell chronic lymphocytic leukemia; Ig, immunoglobulin; Id, idiotype; APC, antigen-presenting cell; FcγR, Fcγ receptor; Ab, antibody; DC, dendritic cell; PBMC, peripheral blood mononuclear cell; mAb, monoclonal antibody; CB, centroblastic lymphoma; CBCC, centroblastic-centrocytic lymphoma; IL, interleukin; HAT, hypoxanthine aminopterin thymidine; FACS, fluorescence-activated cell sorting; FISH, fluorescence *in situ* hybridization; TNF, tumor necrosis factor; ELISPOT, enzyme-linked immunospot.

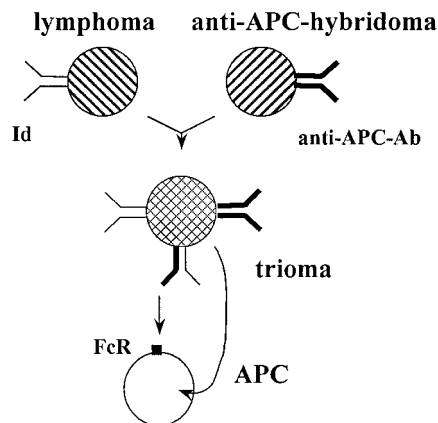


Fig. 1 Principle of the trioma approach. Lymphoma cells are fused to an anti-FcR hybridoma. The trioma cells express lymphoma-derived antigens and the anti-APC specificity. In the mouse model, the lymphoma Id and FcR-binding arm were partly expressed as heterodimeric molecules (5). Binding to FcR leads to uptake and presentation of antigens.

are included in the trioma; (b) targeting of tumor-derived antigens, or possibly whole trioma cells, to FcR-bearing APC; and (c) the xenogeneic nature of the triomas that gives rise to enhanced cross-presentation of antigens (12). Accordingly, vaccination with the purified Ig heterodimer, comprising the Id and one APC-binding arm, that trioma cells express in addition to homodimeric Ig molecules (Fig. 1) was far less efficient in terms of tumor protection than injection of whole trioma cells (5, 12). This differential effect did not reflect the Id-specific Ab response, which was much more pronounced after delivery of Ig protein when compared with cellular vaccines (12, 13). Apparently, humoral immunity is less instrumental for tumor rejection than the T-cell response.

In this study, we extend this approach and demonstrate for the first time that trioma immunization is applicable in the human system. Using *in vitro* assays, we show that an efficient and specific T-cell response can be elicited against B-CLL cells. Therefore, trioma vaccination shows promise as an adjuvant therapy of B-CLL in the clinics.

MATERIALS AND METHODS

Preparation of Tumor Cells and DC. Peripheral blood was obtained from patients after informed consent. PBMCs were isolated by Ficoll-Hypaque density-gradient centrifugation, suspended in RPMI 1640 (Life Technologies, Inc., Karlsruhe, Germany) supplemented with 10% heat-inactivated human serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 units/ml streptomycin; and seeded in six-well plates at a concentration of 2×10^6 cells/well. After incubation for 1 h at 37°C, 5% CO₂, nonadherent CLL cells were removed and used for fusion or frozen for later use. For preparation of DCs, the adherent cells were cultured for 1 week in medium containing 800 units/ml granulocyte-macrophage colony-stimulating factor and 500 units/ml IL-4 (Genzyme, Wiesbaden, Germany). CB and CBCC cells were obtained from lymph node biopsies and depleted of T lymphocytes by using anti-CD6-coated magnetic beads.

Generation of Trioma Cells. The murine hybridoma cell line 197 that expresses an antihuman CD64 Ab (14) was rendered azaguanine resistant. For fusion, this cell line was mixed with tumor cells at a ratio of 3:1 and incubated with 50% polyethylene glycol 1500 (Boehringer Mannheim, Mannheim, Germany) for 1 min. Then the suspension was slowly diluted with 5 ml of PBS (Life Technologies, Inc.). Cells were washed; resuspended in RPMI 1640 supplemented with $1 \times$ HAT supplement (Sigma, München, Germany), 20% human serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 units/ml streptomycin; and plated on 96-well dishes at a density of 10^4 cells/well. Under HAT selection, trioma clones became visible after 7–14 days. They were characterized by FACS, PCR, and FISH.

Flow Cytometry. Expression of anti-CD64 was determined by incubating FcγR-expressing U937 cells with supernatants of hybridoma or trioma cells. Bound anti-CD64 Ab was detected with FITC-conjugated polyclonal goat Ab against mouse IgG (Dianova, Hamburg, Germany). Alternatively, mouse Ig was detected on the surface of trioma cells by FACS analysis of trioma cells labeled with goat antimouse IgG. For phenotyping CLL and trioma cells, the cells were incubated with FITC-labeled Ab against human CD5, CD19, CD20, IgG, and IgM, respectively, for 30 min on ice. Samples were washed and subjected to bidimensional analysis using a FACSCalibur (Becton Dickinson, Heidelberg, Germany). Dead cells were excluded by using propidium iodide (ICN, Eschwege, Germany).

FISH Analyses. Human sequences from trioma clones were amplified by *Alu*-PCR (15). DNA was labeled with biotin-11-dUTP using standard nick translation procedures and hybridized on human 46xy metaphase spreads. Biotin was detected by avidin-labeled Texas Red (1:300). For image acquisition, a Leica DMRXA-RF8 epifluorescence microscope was used, and images were analyzed by using the Leica CGH-Software package (16).

PCR. Fragments from human MHC class I and β₂-microglobulin genes were amplified by PCR. The primer combinations and conditions for this PCR approach were described previously (17).

T-cell Stimulation. To stimulate T cells, 1×10^6 autologous PBMCs or CD14⁺-depleted PBMCs were cultured with 2×10^5 irradiated (100 Gy) trioma, lymphoma, or hybridoma cells, respectively, in 1.5 ml of RPMI 1640 supplemented with 15% heat-inactivated human serum, 2 mM L-glutamine, antibiotics, 50 units/ml IL-4, and 20 units/ml IL-2 in 24-well plates (Nunc, Wiesbaden, Germany). Restimulation was performed after 8 days by adding 1×10^5 irradiated stimulator cells in fresh medium plus supplements. Supernatants for cytokine measurement were harvested after another 8 days.

When responder cells depleted of APC were used in stimulation assays, CD14⁺ cells were eliminated from patient PBMCs by magnetic cell separation using microbeads (Miltenyi Biotec, Sunnyvale, CA). Flow cytometry confirmed the complete lack of the CD14 marker after column purification.

Cytokine Quantitation. The extent of T-cell stimulation was determined by measuring the amounts of T cell-secreted TNF-α and IFN-γ. IFN-γ secreted after stimulation of PBMCs was quantitated by ELISA (PharMingen, Heidelberg, Germany). TNF-α concentrations in the stimulation assays were determined by measuring the cytotoxicity of the supernatants against

Table 1 Characteristics of B-CLL samples used in this study and outcome of fusion and stimulation experiments

Patient	Age	Sex	Binet stage	WBC	% lymphocytes	% CD5/CD19	% CD3	Fusion success ^a	Stimulation index ^b
L.O.S.	64	M	B	14600	67	81	16	—	n.a. ^c
H.P.F.	59	M	C	13100	60	96	3	+	n.d. ^c
A.M.I.	76	F	C	134000	97	97	2	+	n.d.
K.R.A.	53	M	B	117000	92	90	6	—	n.a.
E.K.R.	63	M	C	248000	96	89	2	—	n.a.
A.M.Ü.	69	M	A	131000	94	97	2	+	4.0
M.P.R.	58	M	A	55400	88	93	5	—	n.a.
M.E.I.	61	F	B	23000	75	73	6	+	n.d.
E.J.E.	29	F	A	165000	92	95	4	+	n.d.
S.S.A.	35	M	A	21300	61	n.d.	n.d.	+	2.0
A.S.C.	62	M	B	80000	79	91	8	+	2.5

^a All clones were tested by PCR, FACS, and FISH analyses.

^b Ratio of cytokine secretion in response to CLL cells after trioma priming of autologous PBMCs in comparison with prestimulation with CLL cells alone; stimulations were done in triplicate apart from patient A.S.C. whose PBMCs were stimulated in duplicate.

^c n.a., not applicable; n.d., not determined.

WEHI 164 (18). WEHI cells were suspended at a density of 2.4×10^4 cells/well in 40 μ l of RPMI 1640 with L-glutamine and 10% FCS supplemented with 2 μ g/ml actinomycin D in flat-bottomed 96-well plates. A 40- μ l supernatant from each stimulation assay was transferred to the WEHI suspension. For quantification, a standard titration of TNF- α was included. After incubation for 20 h at 37°C/6.5% CO₂ in a humidified chamber, 20 μ l of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonyl)-2H-tetrazolium/phenazine methosulfate (Promega, Madison, WI; No. G1112) solution (10 mg/ml in PBS) were added to each well. After another 4 h of incubation, 25 μ l of a 10% aqueous SDS solution were added. Then, cell viability as indicated by color development was measured at 492 nm.

Intracellular FACS. For intracellular cytokine staining, T cells from the stimulation assay (1×10^6 cells/ml) were incubated for 6–8 h in the presence of brefeldin A (Sigma) at a final concentration of 1 μ g/ml to block protein secretion. To each sample, FITC-conjugated Ab against surface markers, such as CD3, CD14, and CD19, were added. After 15 min of incubation, cells were fixed and permeabilized using Fix and Perm Kit (Caltag Laboratories, Burlingame, CA) according to the manufacturer's protocol. Staining for TNF- α was performed using phycoerythrin-labeled mouse antihuman TNF- α mAb (Mab11; PharMingen). After 15 min of incubation at room temperature in the dark, 1 ml of FACS buffer was added. Cells were pelleted ($400 \times g$, 5 min), resuspended in 250 μ l of FACS buffer, and examined by flow cytometry.

ELISPOT. The number of tumor-specific cells after stimulation of PBMCs was determined by an ELISPOT assay, as described elsewhere (19). In brief, 96-well plates (Millipore, Bedford, MA) were coated overnight at 4°C with 100 μ l of 15 μ g/ml anti-IFN- γ mAb (clone 1-D1K; Mabtech, Stockholm, Sweden). One-hundred μ l of trioma- or CLL-stimulated PBMCs (2×10^5) were added in triplicates, together with 100 μ l of autologous target cells (2×10^4 CLL cells or DC). After 20 h at 37°C, cells were lysed and incubated for 3 h at room temperature with 100 μ l of 2 μ g/ml biotinylated anti-IFN- γ mAb (clone 7B6-1; Mabtech). Responses were expressed as mean numbers of spots per well after subtracting the mean numbers that were obtained after incubating stimulated PBMCs only with medium.

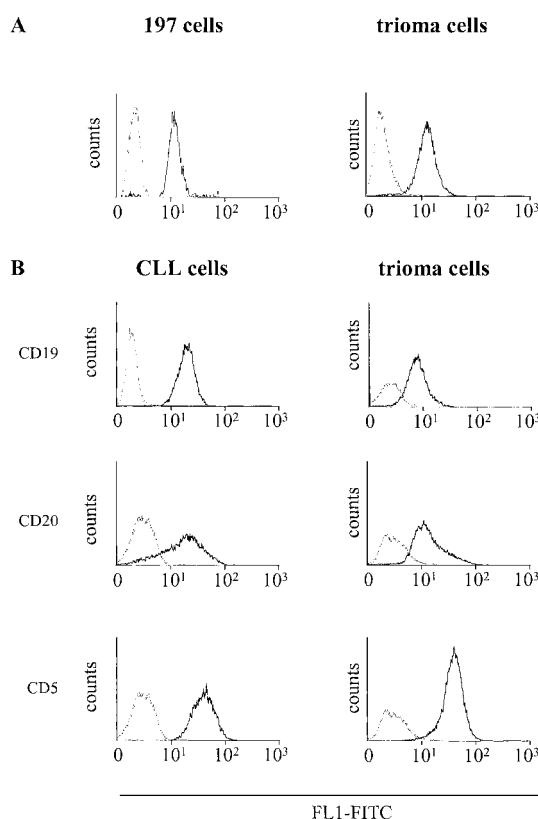


Fig. 2 Characterization of CLL and trioma cells. A, expression of the murine anti-CD64 specificity. Trioma cells or parental hybridoma 197 cells were labeled with FITC-conjugated polyclonal goat antimouse IgG. B, expression of CD19, CD20, and CD5 by tumor cells of a CLL patient and by the corresponding trioma cells. Thin lines, control staining with isotype-matched mAb.

RESULTS AND DISCUSSION

Generation and Characterization of Human Trioma Cells. In preclinical studies designed to evaluate trioma vaccination for the adjuvant treatment of malignant B-cell disorders, we fused B-CLL cells from 11 patients to the murine

Fig. 3 Representative FISH obtained with a normal human metaphase spread and biotinylated *Alu* PCR products of trioma cells from one patient. Hybridization signals are in yellow. No DNA hybridizing to the human metaphases could be amplified from mouse hybridoma cells.

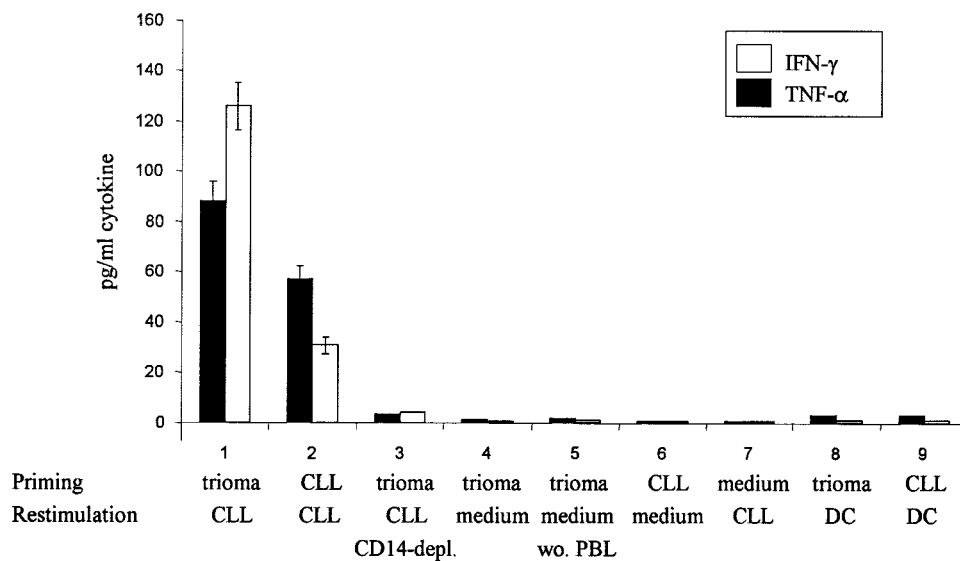
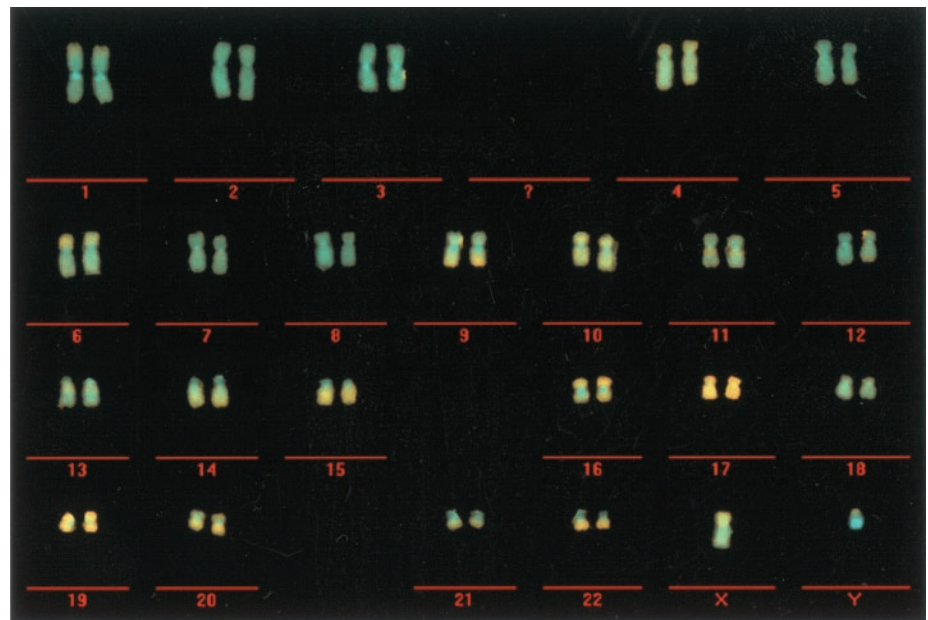


Fig. 4 Activation of autologous PBMC by different stimulator cells. PBMCs of a representative patient were incubated twice with irradiated stimulator cells, as indicated. TNF- α and IFN- γ concentrations in coculture supernatants were determined after the second stimulation round as readout for T-cell activation. The differences between setting 1 and 2 are significant ($P = 0.0002$ for TNF- α ; $P = 0.0007$ for IFN- γ). Each bar shows the mean of three independent stimulations. Maximum activation, which can be obtained by two consecutive trioma stimulations and which represents antileukemia as well as xenogeneic responses, was ~2-fold higher than T-cell activation in the trioma-CLL stimulation setting.

hybridoma 197 that expresses an Ab specific for human Fc γ RI (CD64; Ref. 14). After limiting dilution under HAT selection, we obtained clonal trioma cells in seven cases. These clones can be propagated indefinitely. Patient characteristics and the outcome of the fusion experiments are summarized in Table 1. Because the anti-Fc γ RI specificity is required to target APC, FACS analyses were made to demonstrate the expression of the CD64-specific Ig by the trioma cells. All trioma cell lines expressed the murine Ig, as detected on their surface (example in Fig. 2A) as well as in the supernatants. The second requirement for trioma cells to confer immunity, *i.e.*, the inclusion of tumor-derived components, was verified by FACS, PCR, and

FISH analyses. Human CD5, CD19, and CD20 molecules that were present on the surface of all CLL samples could also be detected on the corresponding trioma cells (example in Fig. 2B). The slight reduction of the expression of surface markers that was observed in some trioma cell lines (CD19 and CD20 in Fig. 2B) might be related to altered gene regulation occurring after fusion. Although IgM and IgG were expressed by most CLL cells, their expression was lost after fusion (data not shown). To verify the presence of human MHC class I molecules, a PCR assay was used because most anti-HLA Ab cross-react with mouse MHC. By using a PCR specific for HLA-A2 and HLA-non-A2 alleles (17), we could detect human MHC class I genes

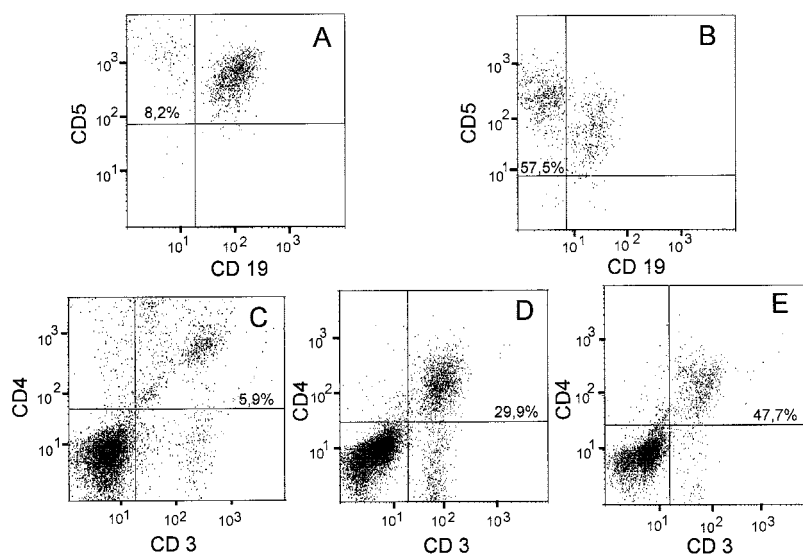


Fig. 5 Proportion of T and B cells in different stimulation assays. A and B, PBMCs of patient A.S.C. were labeled with anti-CD5 and anti-CD19 mAb before stimulation (A) and after sequential stimulation with trioma and lymphoma cells (B). T cells are CD5⁺CD19⁻; leukemic B cells are CD5⁺CD19⁺. C–E, CD3/CD4 staining of naïve PBMC (C), of PBMC after lymphoma–lymphoma stimulation (D), and after trioma–lymphoma stimulation (E). Percentages of CD5⁺CD19⁻ and CD3⁺CD4⁺ cells, respectively, are indicated. Similar results were obtained from all patients whose T cells were subjected to trioma stimulation.

that are localized on chromosome 6 in all trioma cell lines (data not shown). The presence of the β_2 -microglobulin gene that resides on chromosome 15 could also be verified by PCR. To assess the amount of CLL-derived genetic material retained in the trioma cells on a genome-wide scale, we used an *Alu*-specific PCR for isolation of human sequences and hybridized the amplified fragments on human metaphases using FISH. As exemplified in Fig. 3, human DNA sequences could be identified in the trioma cell clones.

T-cell Activation by Trioma Cells. The potential of the trioma cell clones to induce autologous T-lymphocyte responses against CLL cells was tested by *in vitro* stimulation assays. Autologous PBMCs were primed with irradiated trioma or CLL cells, and T-cell activation was determined in response to irradiated unmodified tumor cells by measuring release of TNF- α . Stimulation indices were calculated as the ratios of cytokine release after priming with trioma cells in comparison with priming with tumor cells. To confirm the T-cell origin of the measured cytokine, cells derived from the activated cultures were stained for CD3, CD14, or CD19 and examined for the presence of intracellular cytokine by FACS analysis. Irrespective of the stimulation protocol, CD14⁺ or CD19⁺ cells did never stain positively for intracellular cytokine in contrast to CD3⁺ cells. This indicates that the readout system is a suitable indicator for T-cell activation. In addition, as a second cytokine secreted by T cells, IFN- γ was determined in some cultures. In all experiments, the release of IFN- γ exactly paralleled TNF- α secretion (Fig. 4).

Trioma clones from three patients were included in the stimulation assays. If patients had undergone chemotherapy before, PBMCs were taken after recovery of immune function. Stimulation indices are compiled in Table 1; in Fig. 4, typical results of one CLL patient are exemplified. A single stimulation with either irradiated unmodified lymphoma cells or trioma cells was not sufficient to activate T lymphocytes (Fig. 4, columns 4, 6, 7). In this setting, cytokines released into the supernatants did not exceed background levels that were obtained in the absence

Table 2 Trioma stimulation of PBMCs from non-CLL patients

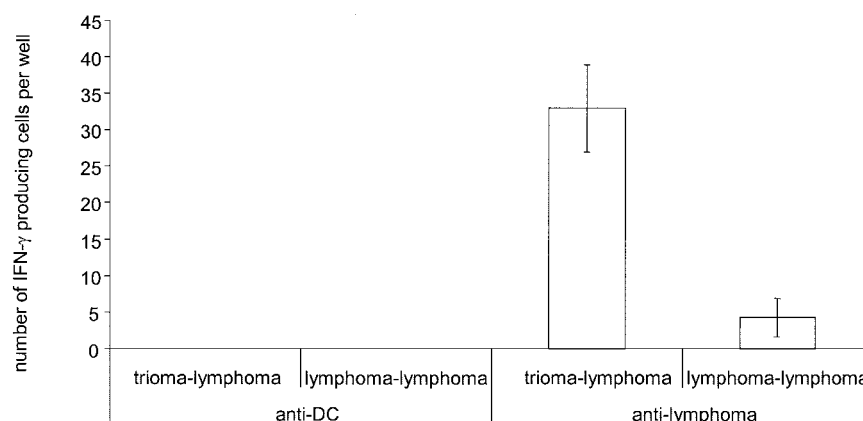
Patient	Age	Sex	Diagnosis	Stimulation index
S.N.E.	37	F	CBCC	1.9
E.B.A.	61	F	CB	4.2

of PBMC (column 5). This indicates that the malignant B cells that were present in the stimulation assays because they could not be quantitatively eliminated from the PBMC preparations were not able to provide stimulation. In contrast, two consecutive stimulations with irradiated tumor cells gave rise to T-cell activation (column 2). However, when restimulation with tumor cells was preceded by a stimulation with trioma cells (column 1) rather than with lymphoma cells (column 2), a significantly higher response was observed. Stimulation indices of 2–4 were found in all samples investigated. This effect is not related to a xenogeneic response because stimulation with trioma cells alone showed no effect (column 4), and subsequent restimulation with lymphoma cells (column 1) excluded reexposure to xenoantigens.

Crucial for the efficiency of trioma-based vaccination in mice were APC, which ingest tumor-derived antigens (11, 12). To demonstrate a similar role for APC in the induction of T-cell responses in the human *in vitro* assays, patients' PBMCs were depleted of CD14⁺ cells before incubation with stimulator cells. As predicted, the effect of trioma cell-mediated activation was completely abrogated when APCs were eliminated from the T-cell preparation (column 3). The finding that effective responses require cross-presentation of antigens by APC was not surprising because trioma cells are derived from CLL cells, and unmodified CLL cells were shown not to be able to efficiently prime T lymphocytes directly (20).

The proportion of T cells before and after stimulation was determined by FACS analyses (Fig. 5). CD5⁺CD19⁻ cells increased from 8% in naïve patient PBMCs to 58% after consecutive stimulation with trioma and lymphoma cells (Fig. 5, A

Fig. 6 ELISPOT assay showing the frequencies of stimulated effector cells reacting with tumor and nonmalignant cells, respectively. 2×10^5 PBMCs from patient A.M.U that had been stimulated twice with irradiated trioma or lymphoma cells as indicated were incubated with autologous DC or CLL cells or with medium alone. Activated cells were visualized by virtue of their IFN- γ secretion. Each bar represents the mean of a triplicate. The numbers of spots were corrected for background levels obtained in the medium controls (average background = four spots). No spots were observed when only CLL cells or DCs were present in the wells.



and B). To define the types of T cells responding to trioma stimulation *in vitro*, we analyzed the ratio of CD4⁺ and CD8⁺ cells recovered from the coculture assays. The ratio of CD4⁺ to CD8⁺ cells increased from 1.8 in naïve PBMCs to 2.4 when stimulated twice solely with autologous CLL cells and to 8 when first stimulated with triomas (Fig. 5, C–E). This bias toward CD4⁺ cells induced by trioma cells may be desirable if not obligatory for therapeutic application, because in our mouse model, tumor-specific CD4⁺ cells isolated from trioma-vaccinated mice were able to eradicate established lymphomas after adoptive transfer in the absence of CD8⁺ lymphocytes (21).

T-cell Induction against Solid Lymphoma. To examine whether the trioma approach is also applicable to other B-cell malignancies than B-CLL, we used malignant B cells that were isolated from lymph node biopsies of two patients with CB and CBCC, respectively (Table 2). Because only these two cases were investigated, it remains open how frequently triomas can generally be derived from patients suffering from these disorders. When autologous PBMCs were subjected to trioma-mediated stimulation, similar stimulation indices were obtained as in CLL patients (Table 2). The experiments also show that the stimulation indices obtained do not depend on diminished or functionally altered T cells, which may occur in peripheral blood of CLL patients.

Tumor Specificity of Trioma-induced T Cells. Although the emergence of autoreactive T cells was never observed after trioma treatment in our mouse model (Refs. 12 and 21),⁴ we were interested to determine whether human T lymphocytes that were stimulated with triomas *in vitro* were tumor specific. We therefore tested trioma-stimulated lymphocytes recognizing autologous CLL cells for their reactivity against autologous nonmalignant cells of the patients. Even those effector cells that were optimally activated to recognize CLL cells through trioma coculture were not capable of recognizing autologous DC (Fig. 4, columns 8, 9). These results could be confirmed in an ELISPOT assay (Fig. 6). Although the absolute numbers of spots were low because of decreased T-cell counts in the patient's blood, the frequency of CLL-reactive T cells was

considerably enhanced after sequential stimulation with trioma and lymphoma cells as opposed to exclusive stimulation with lymphoma cells. Autologous DCs, however, were not recognized in the ELISPOT. Obviously, despite its strong immunostimulatory potential, trioma-dependent T-cell stimulation did not break tolerance against the normal array of self-antigens expressed by DC. Of course, this does not rule out the possibility that T-cell responses to normal B cell-specific components might be triggered *in vivo*. However, blood cell counts of trioma-vaccinated mice indicated that such autoreactive responses did not occur.⁵

Concluding Remarks. Despite recent advances in chemotherapy, most patients suffering from B-CLL will relapse because of the persistence of malignant cells after conventional therapy (22). Trioma vaccination is an immunotherapeutic protocol that allowed complete eradication of malignant lymphoma in a mouse model (5, 11). Here, we show for the first time that this approach is also effective for inducing immunity against malignant B-cell disorders in humans. We demonstrate that trioma vaccination may be a feasible strategy for adjuvant therapy of B-CLL. It is a fairly simple protocol that does not necessitate an adaptation of the malignant cells to cell culture because fusion does not require cycling tumor cells. Trioma cells are clonal cell lines that are defined with respect to markers indicating their CLL origin. Because the trioma cells are immortalized by the fusion, they can be grown rapidly to high cell numbers needed for vaccination, and they can be easily cryopreserved, circumventing possible problems related to genetic instability of the hybrid cells. To avoid injection of xenogenized cells, the trioma approach can also be modified to an *ex vivo* approach for use in the clinic.⁶

Although some tumor-derived genetic material was lost on fusion, the antigens that the trioma cells continued to express were sufficient to induce an immune response *in vitro* in all cases investigated. The loss of the lymphoma Id that was observed in all triomas tested does not pose any obstacle, because Id immunity is not pivotal for T-cell stimulation *in vitro* nor for

⁴ R. Mocikat, unpublished results.

⁵ R. Mocikat, unpublished observations.

⁶ C. Adam and R. Mocikat, unpublished observations.

tumor rejection in murine studies (12). Id-specific responses that could be detected in mice could not be enhanced to provide tumor protection despite the xenogeneic setting. The potency of the trioma approach relies on its polyvalency. Therefore, mutants capable of escaping immune detection are less likely to arise, and Id loss mutants can still be efficiently targeted. At present, the CLL antigens that are recognized by trioma-induced T lymphocytes are still unknown. There are some candidates that are overexpressed in CLL cells, such as Bcl-2 (23). Additional studies will show whether such antigens serve as targets for trioma-induced immunity.

ACKNOWLEDGMENTS

We thank Dr. D. J. Schendel for her ongoing support. Furthermore, we thank Drs. M. Dreyling and B. Frankenberger for critically reading the manuscript and A. Brandl and B. Stadlbauer for skillful technical assistance. We also thank Medarex, Inc. for supplying the hybridoma 197.

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