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Isolation and properties of gene-modified mouse *bcr-abl*-transformed cells expressing various immunostimulatory factors

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B210 cells are murine (BALB/c) cells transformed by bcr-abl fusion gene. After intravenous administration they are capable of inducing leukaemia–like disease in syngeneic mice. From these cells a thymidine–kinase less subline was derived. It was significantly less pathogenic than the parental cells. However, a highly pathogenic clone denoted B210cTK$^-$/cl-2 was isolated from its population. As determined by Western blotting, these cells produced more p210$^{bcr\text{-}abl}$ protein than the parental B210 cells. To successfully transfect these cells a modified electroporation method was introduced. Bicistronic plasmids carrying gene for herpes simplex thymidine kinase (HSV TK) and the gene for either granulocyte-monocyte colony stimulation factor (GM-CSF), interleukin-2 (IL-2) or interleukin 12 (IL-12) were used for the transfection experiments. Gradually, cell lines producing these cytokines were isolated in media supplemented with hypoxantin, aminopterin and thymidine (HAT). All of them were highly sensitive to ganciclovir *in vitro* confirming that the cells produced HSV TK. The genetic modification of B210cTK-/cl-2 was associated neither with the alteration of p210$^{bcr\text{-}abl}$ production nor with any changes in expression of MHC class I molecules. From populations of each of the three lines several cell clones were isolated and tested for the production of the respective cytokines. The original uncloned population and several clones differing in the cytokine production were administered intravenously into mice. All animals survived without symptoms of the disease suggesting that the gene-modification was associated with the loss of pathogenicity.

Key words: CML, Bcr-Abl, HSV TK, cytokines, gene-modified tumour cells, pathogenicity

Chronic myeloid leukaemia (CML) is a malignant disease of the hematopoietic stem cells. The neoplastic cells are characterized by the Philadelphia (Ph+) chromosome, which results from the reciprocal translocation between the chromosomes 9 and 22. As a consequence, the *bcr-abl* fusion gene develops. Its product, most frequently the p210$^{bcr\text{-}abl}$ protein, has a high tyrosine-kinase activity surpassing markedly that exhibited by the wild ABL protein [1, 2]. It is generally accepted that the fusion protein produced plays a key role in the pathogenesis of CML. The recently introduced imatinib-mesylate, a potent inhibitor of the p210$^{bcr\text{-}abl}$-associated tyrosine–kinase activity, has made a considerable progress in the therapy of CML [3, 4]. Still, in the past few years there has nevertheless been an increasing interest in developing immunotherapeutic means for treatment of CML. It has been demonstrated that peptides derived from the fusion zone and covering the fusion point are capable of inducing specific immune responses in CML patients [5, 6] and studies in mouse experimental systems indicated that it is possible to induce immunity against the challenge with the syngeneic, highly oncogenic *bcr-abl*-transformed cells [7–9]. In our laboratory a project aiming to develop therapeutic vaccines against the leukaemia-like disease induced in mice by *bcr-abl*-transformed cells is under way. One of the strategies which we are trying to employ for this purpose is the development of cell-based vaccines expressing a variety of cytokines known to act as enhancers of anti-tumor immunity [10, 11].

Materials and methods

Cell lines and media. Ba-p210 (B210) cells are *bcr-abl*-transformed mouse (Balb/c) cells derived by Daley and Baltimore [12] and have been kindly provided to us by G.Q.Daley (Whitehead Institute, Cambridge Center, MA). Their *in vitro* and *in vivo* properties were described in more detail elsewhere [13,

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14]. In brief, they induce leukaemia-like disease in mice after intravenous administration. One TID_{50} corresponds to approximately 10^4 cells. Thymidine-kinase (TK)-less cells B210TK$^-$ were derived in our laboratory after repeated passages of B210 cells at gradually increasing concentrations of 5-bromo-2-deoxyuridine (BrdU) (Sigma-Aldrich corp., St. Louis, MO). Cells were cultivated in RPMI 1640 medium (Sigma-Aldrich Corp., St. Louis, MO) supplemented with 10% heat-inactivated FCS (PAA Laboratories, Linz, Austria), 4 mM glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C in 5% CO_2 atmosphere. In the case of B210/TK$^-$ the medium was supplemented with BrdU (100 μg/ml). For the selection of cells successfully transfected with herpes simplex virus thymidine kinase (HSV TK) carried by bicistronic plasmids (see below), the RPMI was supplemented with hypoxantin, aminopterin and thymidine (HAT) (HAT Supplement, Gibco, Invitrogen, Carlsbad, CA). 293T cells (kindly provided by J. Kleinschmidt, DKFZ, Heidelberg, Germany) were used as a negative control in Western blotting. Their propagation was the same as in previous experiments [15].

Plasmids. The construction of plasmid carrying HSV TK (denoted pTR-IRES TK) [16] and bicistronic plasmids carrying the genes for HSV TK and either granulocyte-macrophage colony stimulation factor (GM-CSF) (denoted pTR-GM-CSF-IRES-TK) or interleukin 2 (IL-2), (denoted pTR-IL-2-IRES-TK) has been described [15]. The bicistronic pTR-IL-12-IRES-TK plasmid which carries the HSV TK gene and also the gene for mouse interleukin 12 (IL-12) was constructed (Fig. 1). The cDNAs of p40 (1008 bp) and p35 (648 bp) subunits of IL-12 were amplified using PCR, subcloned and sequenced. In the second step genes coding for the p35 and p40 subunits were linked up with the 24 base pair long sequence encoding a cleavage site for the cell endoprotease furin (Gly-Gly-Arg-Gly-Arg-Arg-Gly-Gly), [17]. With the use of XbaI and HindIII restriction enzymes the IL-12 fused gene was inserted into pTR-IRES-TK. Plasmid expressing green fluorescent protein (GFP), denoted pTR-UF2 [18] was used for monitoring transfection efficiency. Plasmids were propagated in E.coli DH5-α (Gibco, Invitrogen, Carlsbad, CA) and purified using Maxi Prep DNA isolation Qiagen kit (Qiagen, Hilden, Germany).

Electroporation. Electroporation was performed using Gene Pulser Electroporation system (Bio-Rad, Hercules, CA). Originally, counts of $5x10^6$ cells suspended in 500 μl of electroporation medium composed of HeBS (0.75 mM Na_2HPO_4, 5 mM KCl, 140 mM NaCl, 6mM glucose, 25mM Hepes, pH 7.05), were placed in 0.4 cm gap cuvette (Bio-Rad, Hercules, CA) and 15 μg of plasmid DNA was added. Electroporation was performed at room temperature (RT) exposing the cells to 250V, 975 μF. The cells were allowed to remain in the electroporation buffer for 10 min, and were then transferred onto tissue culture dishes with 4 ml of media. After 48 hrs of cultivation the cells were spun down and resuspended in the selection media. To optimise the procedure the experimental conditions were gradually modified (see the Results section) with the use of pTR-UF2 plasmid carrying the gene for GFP. The cells transfected with pTR-UF2 plasmid were monitored under fluorescent microscope 24 hrs after electroporation. Subsequently, the transfection efficiency was determined by flow cytometric analysis of GFP expression using Flow Cytometer EPICS XL (Beckman Coulter, Inc., Fullerton, CA). Counts of 5 x 10^5 cells were washed with PBS, resuspended in 0.5 ml PBS, and 10 μg/ml propidiumiodide (PI) was added immediately before the flow cytometric analysis. Living cells were gated and evaluated for GFP expression using WinMDI (version 2.8) software.

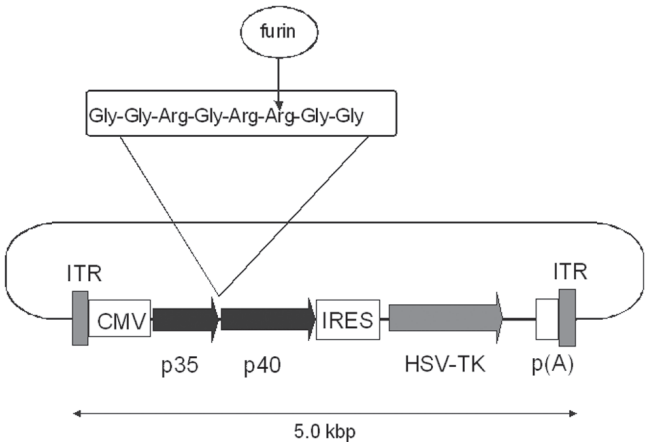

Figure 1. Recombinant plasmid carrying HSV TK and mouse IL-12 genes. It contains the adenovirus-associated virus type 2 (AAV-2) inverted terminal repeats (ITR), the cytomegalovirus immediate early promoter (CMV), mIL-12 gene consisting of p35 and p40 subunits linked with 24 bp sequence containing furin cleavage site, poliovirus type 2 internal ribosomal entry site sequence (IRES), herpes simplex virus thymidine kinase gene (HSV TK) and the bovine growth hormone polyadenylation signal (pA)

GCV sensitivity assay. Counts of 5 x 10^4 cells/well were seeded in 2 ml cultivation media with and without 40 μM GCV (Cymevene, Roche, Basel, Switzerland) and either HAT in 24-well plates. After 5-day cultivation cells were counted using the trypan blue exclusion.

Measurement of cytokine production. Counts of 5 x 10^5 cells were seeded in 3 ml medium in 6-cm culture dishes. The concentration of cytokine, viz mouse GM-CSF, IL2 or IL12, in culture supernatants was measured after 24 hours with the BD OptEIA™ Set Mouse GM-CSF or Mouse IL-2 or Mouse IL-12 (p70) (BD Biosciences, San Diego, CA) following the manufacturer's instructions. Cells were counted using the trypan blue exclusion test. The production level was calculated according to the formula: C/N (where C is the total amount of cytokine in culture medium and N is the final viable cells count) and was expressed in ng/10^6 cells/24 hrs.

Western blotting. Cells were lysed in a lysis buffer (4%SDS, 20% glycerol, 10% mercaptoethanol, 2 mM/l EDTA, 100 mM/l Tris-HCl (pH 8.0)) and after adding bromphenol blue they were boiled for 3 minutes. Lysates of 5 x 10^4 cells were separated by 7% SDS-PAGE electrophoresis. The protein's pattern

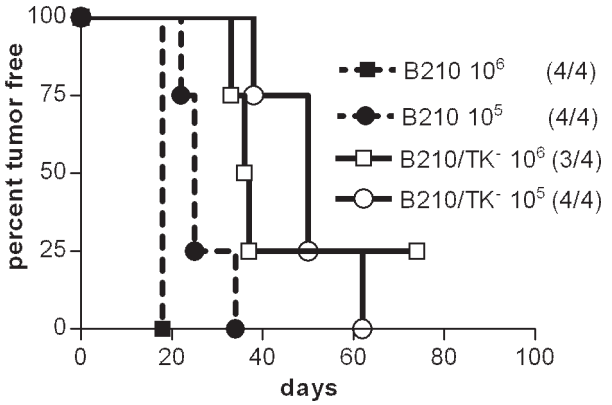

Figure 2. Pathogenicity for mice of B210 cells and its B210/TK- subline

was electroblotted onto nitrocellulose membrane. Any remaining binding sites were blocked in 10% skimmed milk at room temperature for 1 hour. The membrane was then incubated with mouse monoclonal anti-c-ABL antibody (Ab-3, Oncogene Research Products, Boston, MA). The incubation was provided under constant agitation at room temperature for 1 hour and then at 4°C overnight. The membrane was then washed with 0.1% Tween in PBS for 3 x 10 minutes and treated for 1 hour with peroxidase-labelled secondary anti-mouse antibody (Amersham Biosciences, Little Chalfont, UK). The blot was again washed for 3 x 10 minutes. Immunocomplexes were visualized using the ECL plus system (Amersham Biosciences, Little Chalfont, UK).

Flow cytometry for determination of MHC class I and II expression. Counts of 0.5 x 10^6 cells were washed twice with PBS. Detection of MHC class I molecules was performed after 30 min incubation at 4°C (i) with FITC-conjugated anti-mouse either H-2$K^d$$D^d$ monoclonal antibody (Cedarlane, Hornby, Ontario, Canada) or isotype control antibody (Sigma, St. Louis, MO), (ii) with phycoerythrin (PE)-conjugated either anti-mouse H-2L^d monoclonal antibody (Cedarlane, Hornby, Ontario, Canada) or with isotype control antibody (Cedarlane, Hornby, Ontario, Canada), and (iii) with FITC-conjugated anti-mouse either I-A^d monoclonal antibody (Cedarlane, Hornby, Ontario, Canada) or isotype control antibody (Sigma, St. Louis, MO).

Cell cloning. To isolate cell clones from the transduced cells, fresh cultivation medium was mixed with the spent medium, at a ratio 2:1. Spent medium was obtained from the culture of the particular transfected cell type at its growing phase. Before being mixed with the fresh medium, the spent medium was filtered through 0.22μm-Syringe-Filter (TPP, Trasadingen, Switzerland). To isolate cell clones, the transfected cells were diluted to obtain a final concentration of 0.3-cell/0.2 ml. The suspension in 0.2 volumes was distributed into 96-well plate (TPP, Trasadingen, Switzerland). Four hours after seeding all wells were carefully checked for the presence of cells and those containing only one cell were labelled. After approximately 14-day incubation the suspensions from these wells were sucked away and transferred into bigger plates. The cell lines derived were kept frozen in liquid nitrogen until being used.

Animals and oncogenicity assay . Six to 8 week-old female BALB/c mice were obtained from Charles Rivers, Germany. All experiments were carried out in accordance with the Guidelines for Animal Experimentation valid in the Czech Republic. For oncogenicity tests, cells were washed three times with PBS. If not indicated otherwise, counts of 10^6 in 0.2ml volumes of PBS were injected intravenously. Starting one week later, mice were monitored for the symptoms of the disease at least twice a week for up to 100 days.

Statistical analysis. For analysis of the growth curves of the tumours, the two-way analysis of variance was used. Calculations were done using Prism Software Version 3.0 (Graph-Pad Software, San Diego, CA).

Results

Derivation and pathogenicity of cTK- cells. B210 cells were passaged for a prolonged period of time at a gradually increasing concentration of BrdU. Finally, a cell line growing well in the presence of 100 μg of BrdU but incapable of replicating in the HAT media was isolated. The cell line was labelled B210cTK-. To determine its pathogenicity, two different doses (10^5 and 10^6) of these cells were tested in parallel with the parental cells. The results are shown in Figure 2. It can be seen that nearly all mice inoculated with the cTK- cells developed the disease; however, their survival was considerably prolonged ($p<0.01$). This indicated that the loss of cTK, but possibly other mutations which might have been induced by BrdU, resulted in decreasing the virulence of the cells. To obtain more information on the composition of the B210cTK- cell population, we isolated 11 clones and tested 8 of them for leukemogenic potency. The counts of 10^6 cells were administered i.v. The results are shown in Table 1. A marked variation in pathogenicity was apparent. While clone 1-derived cells did not induce disease in any of the mice inoculated, four

Table 1. Pathogenicity of cell clones derived from B210cTK- cells

Clone number	No. of mice with leukaemia/ No. of mice inoculated[1)]
1	0/4
2	3/3
3	2/4
4	3/3
7	3/3
9	3/3
10	2/3
11	2/3

[1)]ninety days after inoculation

other cell lines induced lethal leukaemia in all animals. The earliest onset of the deadly disease was observed in those inoculated with clone 2-cells.All animals died before day 49, while in the case of clones 4, 7 and 9 the last animal died on day 87, 72 and 87, respectively. Since one of the main aims in this study was to find out the impact of cytokine production on the pathogenicity of the gene-modified cells (see below), we selected clone-2 derived cells denoted B210cTK$^-$/cl-2 for further experiments. A large frozen stock of these cells was prepared and in subsequent experiments always the third passage of the thawed cells was used.

Transfection of B210 cells. Our initial attempts to transduce B210 cells using calcium-phosphate precipitation, metafectene (Biontex, Planegg, Germany) or transferin-polyethylenimine (Bender MedSystems, Burlingame, CA) failed. However, the subsequent tests indicated that using electroporation might solve the problem. Our efforts were aimed at optimising the condition of electroporation, i.e. to achieve sufficient percentage of transfected cells and to preserve their survival within acceptable limits. Multiple experiments were performed using pTR-UF2 plasmid carrying the gene for GFP. The efficacy of transfection was monitored by both fluorescent microscopy and flow cytometry. The highest expression of GFP was demonstrated 24 hours after electroporation. Later on, by changing the conditions of electroporation by substituting RPMI 1640 medium for the original elctroporation buffer and exposing the cells to 280-300V and 1050 μF we increased the transfection efficiency up to 16-18% of the surviving cells. The lower capacitance than 1050 μF decreased the transfection efficiency. Higher concentration of plasmid DNA (up to 30 μg) increased the number of GFP-positive cells without producing any dramatic impact on cell survival. The main results are summarized in Tables 2A and 2B. Thus, the optimum condition for electroporation in the present system appeared to be to use RPMI medium and to expose the cells to 280V and 1050 μF at room temperature.

Generation of gene modified B210 cells and their sensitivity to GCV. Based on these results we tried to prepare the cytokine producing B210 sublines. The B210cTK$^-$/cl-2 cells were separately transfected with pTR-GM-CSF-IRES-TK, pTR-IL-2-IRES-TK, pTR-IL-12-IRES-TK or pTR-IRES-TK plasmid, or mock transfected. The transfected cultures were kept in regular cultivation media for 48 hrs and then they were transferred into media containing HAT. Within three weeks marked cell proliferation was detected in all transfected cell cultures, whereas no cells survived in the mock-transfected cultures. To make certain that the cell lines isolated were not revertants to the cTK$^+$ phenotype but real transductants we tested their sensitivity to GCV. At a variance with the parental cells all the transfected cell lines were highly sensitive to GCV, which confirmed the production of HSV TK (results not shown). The transduced cells were labelled B210/2/GM-CSF, B210/2/IL-2, B210/2/IL-12 and B210/2/HSVTK, respectively.

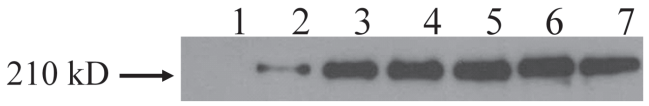

Figure 3. Production of p210$^{bcr-abl}$ in B210 cells and their gene-modified sublines as determined by Western blotting. Anti-c-ABL monoclonal antibody was used. Lane 1: 293T cells (negative control); lane 2: B210 (positive control); lane 3: B210cTK$^-$/cl-2; lane 4: B210/2/GM-CSF; lane 5: B210/2/IL-2; lane 6: B210/2/IL-12 and lane 7: B210/2/HSVTK. Each sample was a lysate from 5 x 10^4 cells.

Cytokine production by gene modified B210 cells. The cell lines were then tested for the cytokine production in ELISA. The B210cTK$^-$/cl-2 and B210/2/HSVTK served as negative controls. The cell lines B210/2/GM-CSF, B210/2/IL-2, and B210/2/IL-12 were confirmed as the producers of the respective cytokines forming 30 ng/10^{6}cells/24 hrs of GM-CSF, 8 ng/10^{6}cells/24 hrs of IL-2 and 160 ng/10^{6}cells/24 hrs of IL-12, respectively.

Immunoblotting detection of the p210$^{bcr-abl}$ protein. The Western blotting test with lysates of parental B210 cells and all the cell lines derived was made to check the expression of p210$^{bcr-abl}$ protein using mouse monoclonal anti-c-abl antibody. The results are shown at Fig. 3. As evident, both the B210cTK$^-$/cl-2 cells and all four transduced cells derived from them produced approximately the same amount of the p210$^{bcr-abl}$ protein, this indicating that the production of the cytokines or HSV TK was not associated with an alteration of p210$^{bcr-abl}$ protein production. However, it is noteworthy that the production of this pro-

Table 2. Influence of varying conditions of electroporation on the expression of green fluorescent protein (GFP) and cell survival

A

El. medium	U (V)	c (μF)	Temperature	% Cell survival	% GFP+
HeBS	250	1050	RT	35	6
PBS	250	1050	RT	30	3
RPMI	250	1050	RT	57	9
HeBS	250	1050	0°C	40	1,2
PBS	250	1050	0°C	28	1
RPMI	250	1050	0°C	45	3,1

B

El. medium	U (V)	c (μF)	Temperature	% Cell survival	% GFP+
RPMI	220	1050	RT	76	3
RPMI	240	1050	RT	62	8
RPMI	250	1050	RT	57	9,6
RPMI	260	1050	RT	60	11
RPMI	280	1050	RT	58	16
RPMI	300	1050	RT	42	18

El. Medium: electroporation medium used, U: voltage, c: capacitance, % Cell survival: percentage of living cells after 24 hrs, %GFP+: percentage of GFP positive cells among living cells after 24 hrs

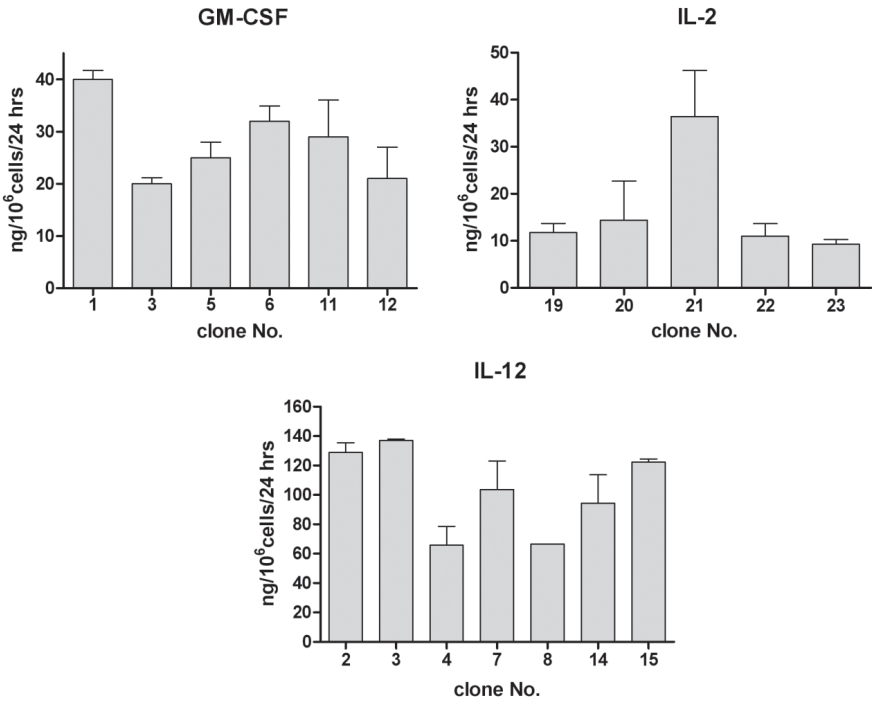

Figure 4. Cytokine production by clones derived from the respective B210 gene-modified cells. All clones were tested simultaneously in three repeated tests.

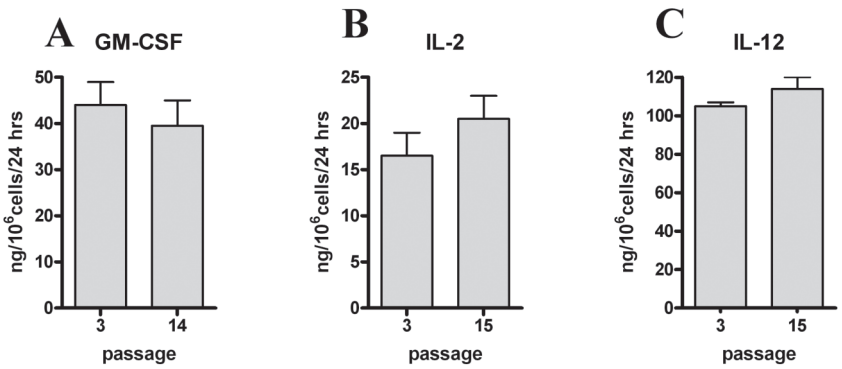

Figure 5. Production of the respective cytokines by the selected cloned cell lines in the course of passages *in vitro*. The following clones were selected: (A) B210/2/GM-CSF/cl-1; (B) B210/2/IL-2/cl-21; (C) B210/IL-12/cl-3

tein by all these cells was considerably higher than in the case of the parental B210 cells used as positive control. Similar results were obtained in all three repeated tests using two different sest of cell lysates.

Isolation of clones of the gene-modified cells and their efficacy as cytokine producers. From the cultures transfected with plasmids carrying the genes for GM-CSF, IL-2 and IL-12 cell clones were isolated and tested for the respective transduced gene products. The results are summarized in Fig. 4. It can bee seen that the production of the cytokines by individual clones markedly differed. The best producers were cultivated in the HAT medium up to fifteen passages, three times a week, and the production of the respective cytokines was measured by ELISA test. It may be seen in Fig. 5 that in the course of passaging the production of the cytokines did not dramatically change.

Determination of MHC class I and II expression of B210 derived cell clones. We also examined parental B210cTK$^-$/cl-2 and the derived cell lines for MHC class I and MHC class II expression. As shown elsewhere [13], MHC class I molecules

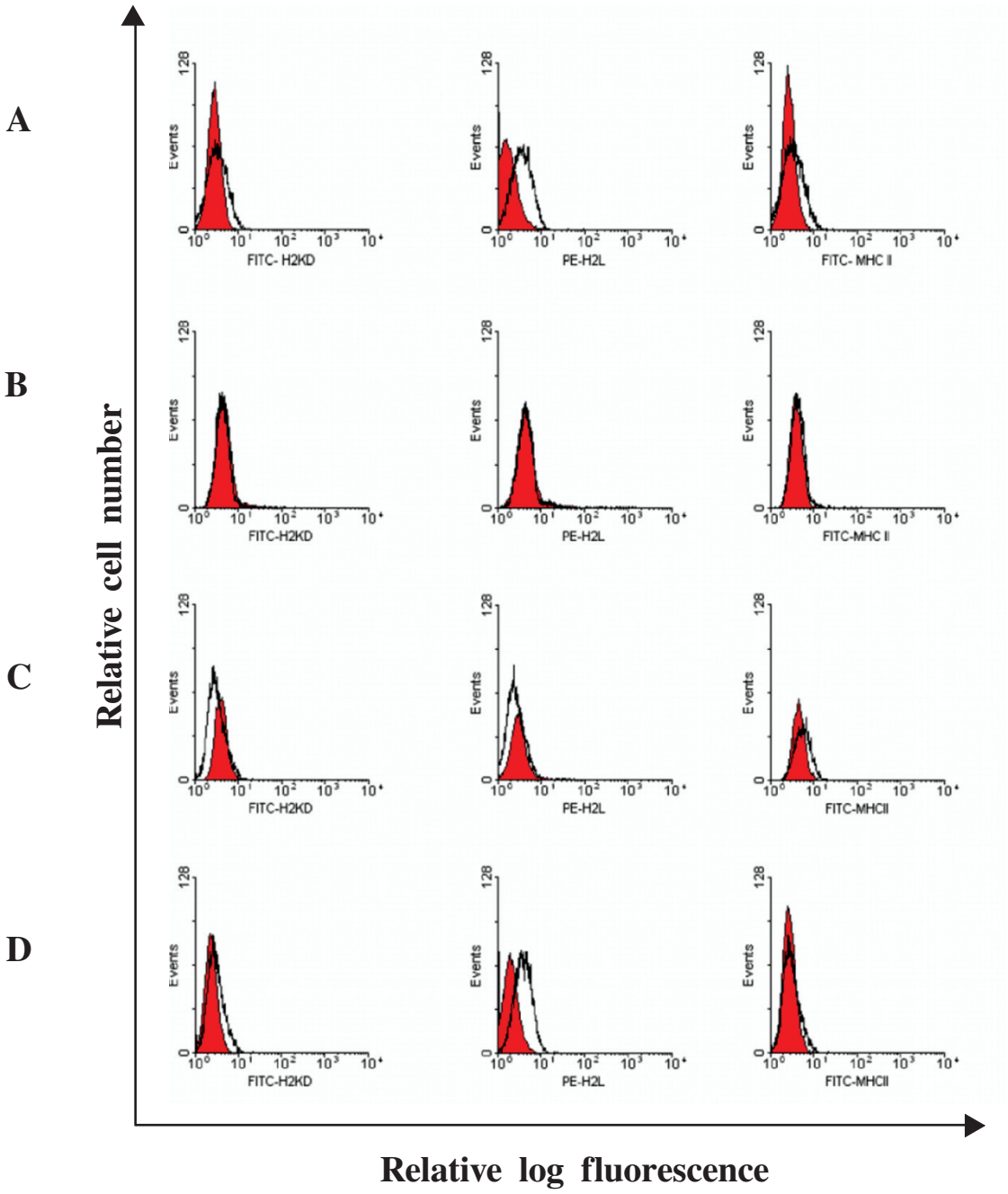

Figure 6. Flow cytometric analysis of MHC class I (H-2K^d and H-2L^d) and MHC class II (I-A^d) expression on gene-modified B210 cells:(A) B210cTK$^-$/cl-2; (B) B210/2/GM-CSF/cl-1; (C) B210/2/IL-2/cl-21; (D) B210/2/IL-12/cl-3. Empty histograms represent cells incubated with isotype control antibodies; filled histograms represent cells incubated with specific antibodies.

in B210 cells tend to be strongly downregulated. Data shown in Fig. 6 provide evidence that this property remained unchanged in all the transduced sublines tested.

Pathogenicity of the gene-modified cells for mice. To examine the influence of the cytokine production on the virulence of the respective cell lines, mice were inoculated with both the uncloned cell populations and with selected cloned sublines expressing either IL-2 or IL-12. In all instances 10^6 cells were administered i.v. The summary of the experiments is in Table 3. It can be seen that none of the cells expressing either GM-CSF or IL-2 or IL-12 was pathogenic for mice. On the other hand, the control cell line expressing

Table 3. Pathogenicity of the gene-modified cells for mice

Cell line	No.of mice with leukemia/ No.of mice inoculated[1)]
B210	4/4
B210cTK$^-$	3/4
B210cTK$^-$ /cl-2	3/3
B210/2/HSV TK	3/4
B210/2/GM-CSF	0/4
B210/2/IL-12	0/4
B210/2/IL-2	0/4
B210/2/IL-12/cl-2	0/3
B210/2/IL-12/cl-3	0/3
B210/2/IL-12/cl-4	0/3
B210/2/IL-12/cl-5	0/3
B210/2/IL-2/cl-19	0/3
B210/2/IL-2/cl-20	0/3
B210/2/IL-2/cl-21	0/3

1)In al instances 10^6 cells were administered intravenously

only HSV-TK induced deadly disease in 3 out of the 4 animals inoculated.

Discussion

In the present experiments the preparation of cytokine-producing *bcr-abl*-transformed cells was undertaken with the use of a system that had proved efficient in our previous experiments. This approach is based on the isolation of cTK-less cells and on making the use of bicistronic plasmids that carried together with the gene of interest also the HSV TK gene [15, 16] for transfection. To derive B210cTK$^-$ was not an easy task. For some reason it took about half a year of continuous propagating these cells at gradually increasing concentrations of BrdU, before cells growing at the 100 μg of this drug were isolated. These cells were less pathogenic than the parental cells. Clonal analysis revealed that the B210cTK$^-$ population was composed of cells mutually markedly differing in their pathogenicity for mice. Since we wanted to determine the impact of the production of selected cytokines on the pathogenicity of the transduced cells, we selected for the transfection experiments a clone of B210cTK$^-$ cells with the highest leukemogenic activity. Our attempts to use for transfection of the B210 cell progenies the techniques, which in our hands had been highly efficient for transfection of epithelial or fibroblastic cells [15, 19, 20], failed completely. However, we were quite successful when using electroporation technique. The optimal conditions for the transduction were defined using a plasmid that carried the gene for GFP. The highest transfection efficiency as well as the best cell survival was achieved with serum-free RPMI medium being used as the electroporation buffer. The transduced cells were producing reasonable amounts of the respective cytokines. The $p210^{bcr-abl}$ production by all transduced cells was approximately the same as detected in the B210cTK$^-$/cl-2 cells from which they were derived. Surprisingly, it was more efficient than by the parental B210 cells. At this writing, no reasonable explanation can be offered for this observation. We can only speculate that this phenomenon seen in repeated tests might be associated with mutations induced in the course of prolonged cultivation of the B210 cells in the presence of BrdU. On the other hand, the transduced cells did not differ from the parental cells in the expression of MHC class I and II molecules. Still, clonal analysis of the population of the transduced cells demonstrated a quite extensive inhomogeneity of the respective cell populations: the cell clones isolated differed widely in the production of all three cytokines. The cytokine production was apparently a stable property of the clones in the course of repeated passages *in vitro*. Since it was difficult to examine all the derived cell lines for their pathogenicity in mice, in addition to the uncloned populations we tested only several clones derived from B210/2/IL-2 and B210/2/IL-12 cells, which differed in cytokine production. All three cell lines were free of leukemogenic activity and also none of the cell clones tested was pathogenic, this suggesting that in none of them the production of the respective cytokine was below the critical level. Experiments are under way to further clarify this point. On the other hand, cells expressing HSV TK were capable of inducing leukaemia in mice, this indicating that the expression of this enzyme did not play any major role in the loss of pathogenicity observed in the case of cytokine-producing cell lines. The successful "attenuation" of the B210cTK$^-$/cl-2 cells provided us with a means useable as live vaccines in the therapy of experimental disease induced in mice by *bcr-abl*-transformed cells. Experiments are under way in which the potency of these vaccines for both prophylactic and therapeutic purposes is tested.

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