Urol Res (2002) 30:233–239
DOI 10.1007/s00240-002-0268-z

ORIGINAL PAPER

Henrik Suttmann · Dieter Jocham
Andreas Böhle · Sven Brandau

The role of LFA-1 in the lysis of bladder cancer cells by bacillus Calmette-Guérin and interleukin 2-activated killer cells

Received: 14 November 2001 / Accepted: 29 November 2001 / Published online: 5 July 2002

Abstract Activated cytotoxic effector cells such as bacillus Calmette-Guérin (BCG)-activated killer (BAK) and lymphokine-activated killer (LAK) cells are thought to mediate the antitumor effects in the immunotherapy of superficial bladder cancer with BCG. We investigated the role of the leukocyte-function-antigen-1 and its two subunits CD11a and CD18 in the lysis of bladder tumor cells by both effector cell populations. We used flow cytometry to measure CD11a and CD18 expression on BAK and LAK cells. The involvement of both surface molecules in the lysis of bladder tumor cells was determined by phase contrast microscopy and a set of radioactive-release assays using specific inhibitory antibodies. BAK and LAK cells expressed more CD11a and CD18 on their surfaces than unstimulated peripheral blood mononuclear cells. Effector-target cell adhesion was a prerequisite for the cell-mediated cytotoxicity of BAK and LAK cells against bladder tumor targets. Cytotoxicity of both BAK and LAK cells was inhibited by a combination of anti-CD11a and -CD18 monoclonal antibodies. Our study gives further insight into the complex interactions of the adhesion molecules of activated immune cells and their respective tumor targets and might help to increase our knowledge of the molecular mechanisms of BCG-immunotherapy.

Keywords Bladder cancer · BCG · IL-2 · LFA-1 · Cellular cytotoxicity

H. Suttmann · D. Jocham · A. Böhle
Department of Urology,
Medical University of Lübeck, Germany

S. Brandau (✉)
Division of Immunotherapy,
Research Center Borstel,
Parkallee 26b, 23845 Borstel, Germany
E-mail: sbrandau@fz-borstel.de
Tel.: +49-4537-188347
Fax: +49-4537-188347

Introduction

Intravesical immunotherapy with bacillus Calmette-Guérin (BCG) is the most effective treatment to prevent bladder cancer recurrences after transurethral tumor resection [26, 31]. In spite of being considered the most successful immunotherapy today, the immunological mechanisms remain only partially understood [1]. The instillation of BCG into the bladder provokes a complex inflammatory response. Lymphocytes and various cytokines are released into the urine [1, 7, 15, 37]. The bladder wall is infiltrated by mononuclear cells such as macrophages, $CD4^+$ and $CD8^+$ T lymphocytes which form granulomas in the suburothelial stroma [6, 19]. In vivo studies with mice have demonstrated that $CD4^+$, $CD8^+$ and NK cells are required for the bcg-mediated antitumor effect [12, 33, 34]. Therefore, cell-mediated immunity plays a key role in the elimination of tumor cells after BCG instillation.

The in vitro cytokine stimulation of lymphocytes with IL-2 generates a strong cellular cytotoxicity against a large variety of tumor targets [36]. These cells have been termed lymphokine-activated killer (LAK) cells and have been proposed as possible effector cells in BCG-immunotherapy [38]. We previously demonstrated the generation of tumor-cytotoxic effector cells by stimulation with BCG [40]. These BCG-activated killer (BAK) cells exhibit a strong cytotoxicity against bladder cancer cells. Further studies demonstrated that BAK cells differ from LAK cells phenotypically and functionally [8, 18]. BAK cell induction involves monocytes and $CD4^+$ T cells as well as cytokines such as IL-2, IL-12 and IFN-γ. BAK cells belong to a small subset of activated $CD3^-/CD8^+/CD16^+/CD56^+$ NK cells and lyse bladder tumor cells via a perforin-mediated pathway [9, 11].

Adhesion molecules are crucially important for the interaction of leukocytes with other cells. Among others, those of the leukocyte-function-associated antigen-1 (LFA-1) have been implicated in the lysis of cancer cells

by LAK and NK cells [2, 42]. LFA-1 consists of a 185 kDa α-chain (CD11a) and a 95 kDa β-chain (CD18). It interacts with various members of the intercellular adhesion molecule (ICAM) family and is expressed on most leukocytes [39]. Its main ligand is the ICAM-1 molecule, a 90 kDa cell surface glycoprotein. LFA-1 is expressed on LAK and NK cells [24, 43]. Furthermore, it has been shown that most bladder cancer cells express adhesion molecules of the ICAM family [14].

To further analyze the molecular mechanisms of BCG-immunotherapy, we tried to define the adhesion molecules involved in the process of cell-mediated cytotoxicity of BAK and LAK cells against bladder cancer cells. We used inhibitory antibodies in a cellular cytotoxicity assay system to block tumor cell lysis. Our results demonstrate an important role for the interaction of LFA-1 with integrins on the target cell surface in this process.

Materials and methods

Cell lines

The human bladder carcinoma cell line T24 was maintained in RPMI-1640 medium (Biochrom, Berlin, Germany) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin at 37°C and 5% CO_2. It was originally established from a patient with a poorly differentiated G3-tumor [13] and kindly provided by L. de Boer (University of Amsterdam, The Netherlands).

Effector cells

Peripheral blood mononuclear cells (PBMC) from healthy human donors were isolated by density-gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden). Cells were adjusted to a concentration of 10^6/ml in RPMI-1640 containing 5% human AB serum, 100 U/ml penicillin and 100 µg/ml streptomycin. We used 200 U/ml human native IL-2 (kindly provided by H. Mohr, Blood Transfusion Service, Springe, Germany) and 3.75×10^4 cfu/ml reconstituted lyophilized BCG (Connaught substrain, Immucyst) to stimulate PBMC. Stimulated cell suspensions were cultured in six-well microtitre plates for 7 days at 37°C and 5% CO_2. In all experiments, unstimulated cultured PBMC served as controls.

Cytotoxicity assay

Cytotoxicity was determined using a L-[^{3}H] methionine release assay in a modification of the method described by Leibold and Bridge [29]. In brief, target T24 cells were labeled for 16 h with L-[^{3}H] methionine (Amersham Buchler, Braunschweig, Germany; specific activity 70–85 Ci/mmol, 20–40 µCi/10 ml) in L-methionine free RPMI 1640 medium (Biochrom) supplemented with 10% FCS, penicillin and streptomycin, at 5×10^3 cells/200 µl in a 96-well microtiter plate (Falcon, 3072, Becton Dickinson, Heidelberg, Germany). Following this step, medium was renewed and effector cells were added at different effector/target (E/T) cell ratios in 200 µl as stated in the figure legends. The inhibitory antibodies anti-humanCD11a (clone 25.3.1) and anti-humanCD18 (clone 7E4, both Coulter Immunotech) were added to the effector cells (concentrations as indicated in the figure legends) 30 min prior to coincubation with target cells to allow binding to their respective antigens. After 20 h of coincubation, 100 µl supernatant was harvested. To determine the spontaneous release, the target cells were cultured in medium alone. Maximum release was measured after the complete lysis of targets in 2% SDS/1% Triton X-100/10 mM EDTA. Specific lysis was determined according to the following formula:

$$\text{specific lysis}(\%) = (r_{exp} - r_{spon}) \times 100/(r_{max} - r_{spon}),$$

with r_{exp}= experimental release; r_{spon}= spontaneous release; r_{max}= maximum release.

"Cold" target inhibition assay

This assay system was developed and described by de Landazuri and Herberman and Berke et al. [4, 16]. We used it to demonstrate that the cell-mediated lysis of bladder carcinoma cells by BAK cells depends on effector-target cell adhesion. Increasing amounts of unlabelled (cold) competitor cells are added to a fixed number of radioactively labelled (hot) target cells prior to coincubation with the effector cells. If effector-target cell binding is necessary for the induction of cellular cytotoxicity, the cold competitors compete with the hot targets for the binding of the effector cells. Since the killing of the cold competitors can not be measured in the radioactive release assay, increasing numbers of cold cells lead to a decrease in the radioactive release and specific cytotoxicity of the hot cells. Increasing numbers of up to 125×10^3 unlabelled competitor cells/well were added to 5×10^3 radioactively labelled target cells before coincubation with the effector cells and allowed to adhere for 4 h. Following this step, effector cells were added and specific lysis was determined as described above.

Flow cytometry

Single-colour immunofluorescence was performed on BAK and LAK cells as well as unstimulated PBMC indirectly stained with monoclonal antibodies and detected with a fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody. For each analysis, 5×10^5 cells were incubated in washing buffer (PBS containing 0.1% sodium azide and 3% human AB serum) with 2 µg/ml anti-CD11a, anti-CD18 or an isotype control at 4°C for 30 min. We used the same set of anti-CD11a and anti-CD18 antibodies for flow cytometry and inhibitory functional assays in accordance with the manufacturer's instructions. After washing twice, a goat anti-mouse-FITC conjugate was added for another 30 min at 4°C. Following two more washes in washing buffer, 1.5% formaldehyde was added and a total of 10,000 cells were analyzed on a FACStar Plus (Becton Dickinson). The gating of debris, non-viable and aggregated cells was done according to forward/sideward scatter signals. We calculated our data with WinMDI software.

Phase contrast microscopy

A total of 5×10^5 target T24 cells were seeded into each well of a 96-well microtiter plate (Falcon, 3072, Becton Dickinson) and allowed to adhere for 6 h in RPMI-1640 containing 5% human AB serum, 100 U/ml penicillin and 100 µg/ml streptomycin. Afterwards, stimulated effector and control cells were added at an effector-/target-cell ratio of 40:1. After an additional coincubation period of 8 h, phase contrast micrographs were taken at 100× magnification.

Results

Dependence of BAK and LAK cell cytotoxicity against bladder tumor cells on effector-target cell adhesion

BAK and LAK cells exerted strong cytotoxicity against T24 bladder tumor cells (Fig. 1a). We used a cold-target

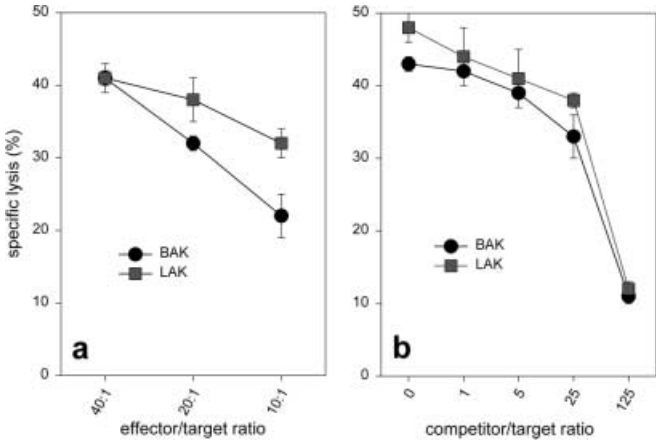

Fig. 1a, b. Lysis of bladder tumor targets by bacillus Calmette-Guérin (BCG)-activated killer (*BAK*) cells and lymphokine-activated killer (*LAK*) cells depends on cell-cell contact. Peripheral blood mononuclear cells (PBMC) were stimulated with BCG and interleukin-2 (IL-2) for 7 days to generate BAK and LAK cells. **a** Cytotoxicity against T24 bladder tumor cells was measured in a radioactive-release assay. Both effector cell populations exhibited strong cytotoxicity against the targets tested. **b** "Cold" target inhibition of BAK and LAK cell killing of bladder cancer cells. A total of 5×10^3 T24 cells were radioactively labelled (hot) and specific lysis was determined in the presence of increasing numbers of unlabelled (cold) inhibitors. Both target cell populations compete for the binding of the effector cells, thus demonstrating the requirement for cell-cell contact and adhesion in the killing process. The effector/target ratio was 40:1. The lysis of unstimulated cultured PBMC (control) was below 5%. Experiments were done in triplicate with results shown as mean ± SD

inhibition assay to investigate whether the lysis of bladder tumor cells by both effector cell populations depends on the adhesion of the effector cells to the target cells. In our experiments, increasing amounts of unlabelled T24 cells competed with labelled radioactive T24 targets for the binding of both effector cell populations and inhibited cytotoxicity as shown in Fig. 1b. Specific lysis was strongly reduced, showing that effector-target cell binding is indeed a prerequisite for cytolysis by both BAK and LAK cells. The process of effector-target cell adhesion and target cell lysis was also observed by phase contrast microscopy. Unstimulated PBMCs do not adhere to bladder tumor cells and target cells remain viable (Fig. 2a). BAK and LAK cells, however, adhere to their respective tumor targets which results in target cell destruction (Figs. 2b, c).

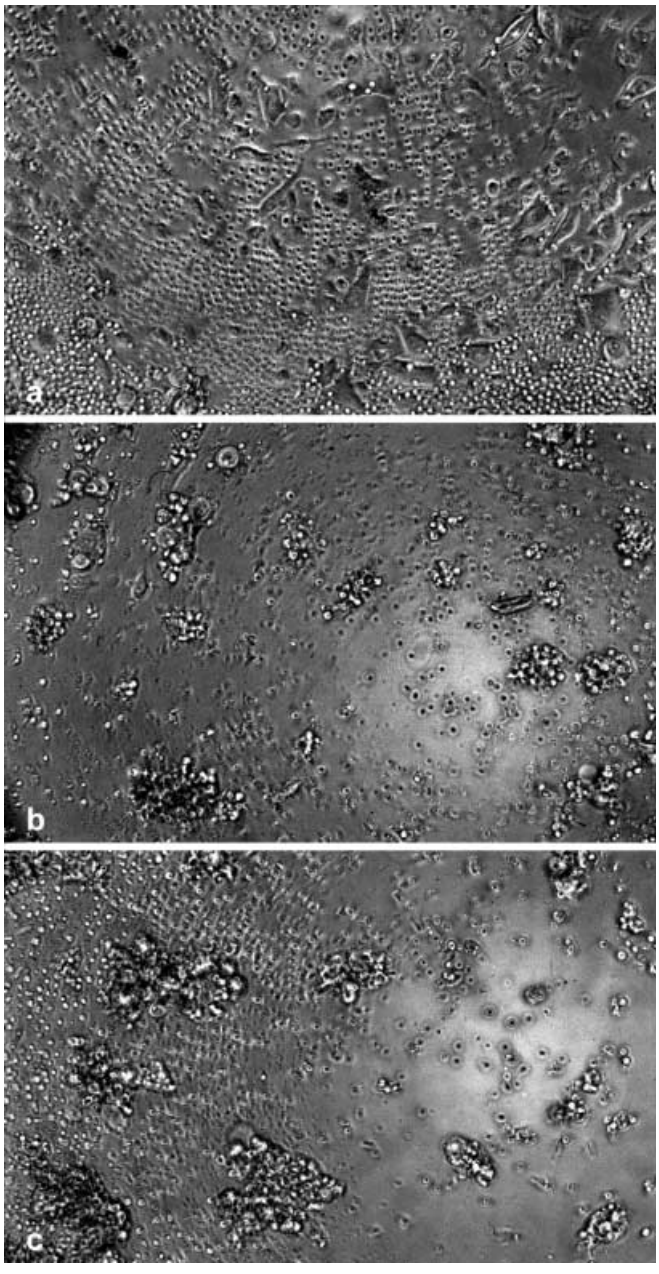

Fig. 2a–c. Effector-target cell adhesion and destruction observed by phase contrast microscopy 8 h after coincubation (100×; E/T-ratio 40:1). **a** Unstimulated PBMCs were scattered around viable T24 bladder tumor cells. There was no adhesion to the target cells. In contrast, BAK (**b**) and LAK (**c**) cells adhere to T24 cells and form clusters around the tumor targets. There were no remaining viable T24 cells

CD11a/CD18-expression on BAK cells, LAK cells and unstimulated PBMC

Cytokine stimulation of PBMC up- or downregulates the expression of various surface molecules including adhesion molecules. We used flow cytometry to determine the expression of CD11a/CD18 on BAK cells compared to LAK cells and unstimulated PBMC. Since CD11a and CD18 are expressed on almost all leukocytes, we used the "mean fluorescent intensity" to evaluate the stimulatory effect of BCG and IL-2. Stimulation of PBMC with BCG slightly increased the expression of CD11a and CD18 (means = 107.9 and 98.8, respectively) as compared to the unstimulated cells (means = 74.4 and 64.6). In contrast, adhesion molecule expression on IL-2-stimulated cells was further increased to a mean of 381.9 and 360.4 for CD11a and CD18, respectively. Table 1 summarizes these results together with the respective percentages of positive cells.

Table 1. CD11a and CD18 expression on BAK and LAK cells. PBMC were stimulated with BCG and IL-2 for 7 days to generate BAK and LAK cells. Both effector cell populations were stained with mouse anti-CD11a and -CD18 mAb or an isotype control and subsequently detected with a fluorescein-isothiocyanate-conjugated mAb. The results were compared with the unstimulated control PBMC. BAK and LAK cells show an increased expression of CD11a and CD18

	% Positive cells			"Mean" over all cells		
	CD11a	CD18	Isotype	CD11a	CD18	Isotype
BAK	94	96.03	2.7	107.9	98.8	13.5
LAK	99.4	95.7	5.6	381.9	360.4	8.78
Unstimulated	96.5	96.6	0.9	74.4	64.6	6.5
95%-confidence interval	6.7	1.1	5.9			

CD11a/CD18 play a crucial role in BAK cell cytotoxicity against bladder tumor cells

Having demonstrated an elevated expression of CD11a and CD18 on BAK and LAK cells, we wanted to define whether these surface adhesion molecules play any role in the process of cell-mediated cytotoxicity against bladder tumor cells. The expression of the most important ligand ICAM-1 on T24 cells has been shown by us (data not shown) and others [14, 20]. Inhibitory anti-CD11a and anti-CD18 antibodies alone or in combination were added to the cytotoxicity assay system and specific lysis was determined. Increasing concentrations of up to 5 μg/ml anti-CD11a and anti-CD18 effectively inhibited BAK cell lysis of T24 bladder tumor cells by as much as 24% and 19%, respectively. However, by adding both antibodies in combination lysis was further reduced by 34% (Fig. 3a). Thus, blocking the LFA-1 molecule as a whole has a different functional effect compared to blocking each subunit separately. Additionally, we used the same experimental setup to inhibit LAK cell lysis of T24 cells by anti-CD11a and anti-CD18 antibodies. Maximal concentrations of 5 μg/ml anti-CD11a and anti-CD18 reduced LAK cell cytotoxicity by 13% and 19%, respectively. The combination of both antibodies did not decrease cytotoxicity any further. With 5 μg/ml anti-CD11a and anti-CD18 together, an inhibition of 19% was attained (Fig. 3).

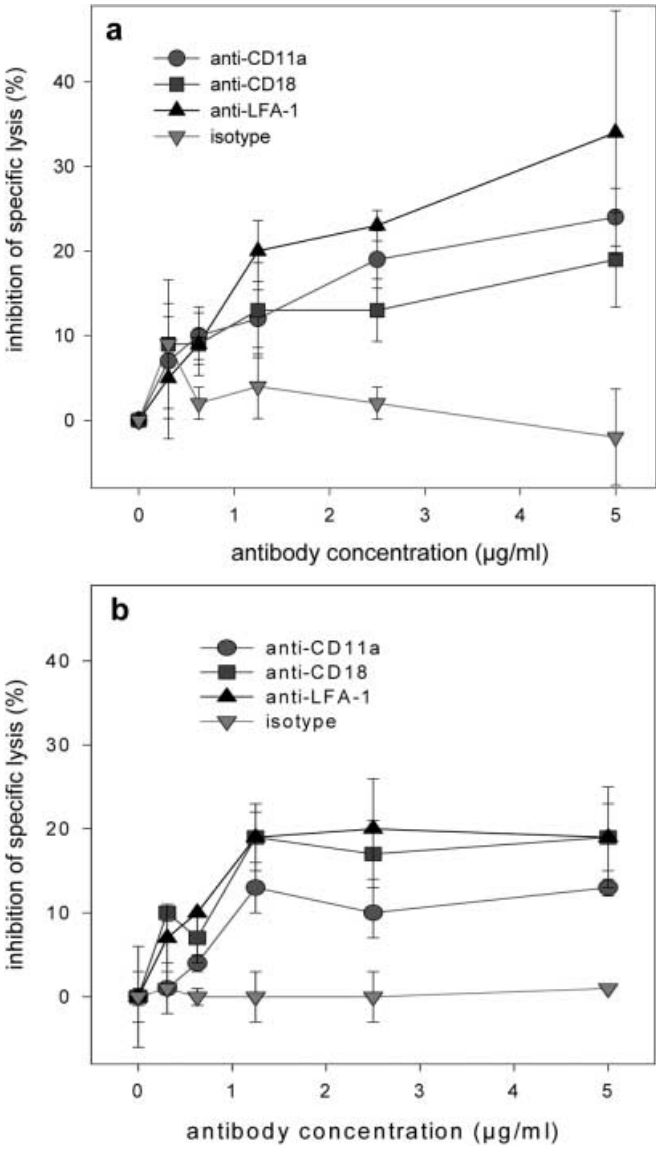

Fig. 3a, b. Inhibition of BAK and LAK cell-mediated cytotoxicity against bladder tumor cells by inhibitory anti-CD11a and -CD18 mAb. PBMC were stimulatad with BCG and IL-2 for 7 days to generate BAK and LAK cells. Antibodies were added to effector cells 30 min prior to coincubation with target cells to assure optimal antigen binding. Specific lysis of T24 bladder turmor targets was determined in the presence of increasing concentrations of anti-CD11a and -CD18 as well as an isotype control. **a** BAK cell cytotoxicity was inhibited by both antibodies. The combination of both antibodies led to a further decrease of specific lysis. **b** LAK cell cytotoxicity was inhibited by both antibodies. The combination did not result in a further decrease of specific lysis. The effector/target ratio was 40:1. The lysis in the controls was below 5%. Experiments were done in triplicate with results shown as mean ± SD

Discussion

Much effort has been put into the development and improvement of immunotherapeutic strategies for the treatment of urological malignancies. Intravesical instillation of BCG for the prevention of superficial bladder cancer recurrences is considered the most effective immunotherapy today [26, 31]. BCG has been shown to be superior to chemotherapeutic drugs used for the local treatment of bladder tumors [23]. Activated immune cells are thought to mediate the antitumor effects of BCG in vivo [12, 33, 34]. In vitro, IL-2 and BCG are potent inducers of cell-mediated cytotoxicity against various human tumor targets [10, 22, 40]. LAK cells can be generated in vivo and directly by cytokine stimulation of PBMC or isolated lymphocytes [3, 22]. However, there is a large variety of possible LAK cell phenotypes [32]. LAK cells can express NK cell markers

such as CD16 and CD56 as well as T cell markers such as CD3 and CD8. In contrast, the generation of BAK cells involves a complex interaction of various PBMC subsets with cytokines. Monocytes and $CD4^+$ T cells as well as cytokines such as IL-2, IL-12 and IFN-γ are required for their induction [35, 41]. BAK cells belong to a small activated NK cell subset and are of the $CD3^-$/$CD8^+$/$CD16^+$/$CD56^+$ phenotype [9]. Both effector cell populations lyse their targets in an MHC-unrestricted manner [3, 40]. Still, little is known about the direct cell-cell interactions and adhesion processes between BAK and LAK effectors and their respective targets. While many in vitro studies have been conducted recently to identify the adhesion molecules involved in LAK cell lysis, we also tried to define those important for BAK cell cytotoxicity against bladder tumor cells.

Two ways for cytotoxic effector cells to induce target cell lysis have been described: they either release soluble cytotoxic factors or form effector-target cell conjugates to initiate specific target cell destruction [4]. Much progress has been made in elucidating the molecular mechanisms of cytolysis used by BAK and LAK cells. While LAK cell lysis is mediated through FasLigand, perforin and TNF-α, BAK cells kill predominantly via perforin [11, 28]. Effector-target cell adhesion is a prerequisite for perforin- and FasLigand-mediated cytolysis. We were able to show in cold-target inhibition assays that the BAK and LAK cell lysis of bladder tumor targets (Fig. 1a) depends on the establishment of cell-cell contact (Figs. 1b, 2a-c). LFA-1, with its two subunits CD11a and CD18, is the major integrin on lymphocytes [21, 27]. Therefore, we further analyzed the role of these adhesion molecules in BAK and LAK cell lysis. As pan-leukocyte markers, about 95% of both effector cell populations express CD11a and CD18 on their cell surfaces. However, there was a great difference in the mean fluorescent activity (Table 1), demonstrating a far lower expression of both molecules on BAK cells in comparison with LAK cells. An increased expression of LFA-1 on LAK cells has been described previously [25, 30]. Using inhibitory antibodies against CD11a and CD18 we were able to inhibit cytotoxicity of BAK cells against T24 by up to 34% (Fig. 3a). Thus, both adhesion molecules play an important role in this process. Using the same experimental setup, LAK cell lysis was inhibited by 19%, indicationg a role for LFA-1 as well in LAK cell cytotoxicity (Fig. 3b). The involvement of LFA-1 in LAK cell lysis of T24 tumor cells has already been investigated by Campbell et al. with a somewhat different result [14]. By blocking LFA-1, they reduced adhesion by 75% and cytotoxicity by about 60%. Jackson et al. were able to inhibit LAK cell lysis of other bladder tumor cell lines by about 65% using antibodies against LFA-1 [24]. In their experiments, LAK cell cytotoxicity was positively correlated with the expression of ICAM-1, the most important LFA-1-ligand, on the target cell surface. Donskov et al. used anti-CD11a and -CD18 antibodies in a murine tumor model to inhibit LAK cell lysis. Neither antibody had any effect on specific cytotoxicity. However, by combining the antibodies they found a reduction in lysis of more than 50% [17]. In our own experiments, we observed a similar effect with the BAK cells. Anti-CD11a and -CD18 reduced the killing of T24 cells by 24% and 19%, respectively. The combination of both antibodies resulted in a further decrease, inhibiting cytotoxicity by 34%. We were not able to detect a similar effect in our experiments with the LAK cells. Another interesting observation was made by Blanchard et al. [5]. By using anti-CD18 antibodies they were able to inhibit the cytolysis of tumor cells by LAK cells. However, the adhesion of the effector cells to their target cells remained unaffected. By blocking CD11a, both cytolysis and adhesion were inhibited. The authors speculated that CD18 might be involved in transducing an apoptotic or cytotoxic signal, while CD11a might be involved mainly in mediating adhesion.

There are various protocols to generate LAK cells, and in all studies mentioned above, a different protocol has been used, which might account for some of the differences observed. In addition, every group used a different set of CD11a/CD18 antibody clones. The ICAM-1 expression, as well as the expression of various other integrins on the target cell surface, might also influence the binding and function of the respective antibody on the effector cell side. Further studies will be needed to clarify the role of CD11a and CD18 in the lysis of tumor cells by various effector cell populations.

BAK and LAK cells have been implicated in the mode of action of BCG immunotherapy. Effector-target cell adhesion is a prerequisite for tumor cell elimination by various cytolytic effector cell populations. Cancer cells are known to escape intrinsic tumor control by changing the expression of the surface adhesion molecules. Therefore, an understanding of the molecular interaction between effector and tumor target cells is of crucial importance for the development and improvement of therapeutic strategies. This study demonstrates a role for the LFA-1 interaction with ICAM-1 in the lysis of bladder tumor cells by BAK and LAK cells. Tumor cell lysis in both populations depends on the establishment of cell-cell contact. CD11a and CD18 are important in BAK and LAK cell cytotoxicity against T24. As a powerful modulator of the immune system, BCG alters the expression of adhesion molecules on activated immune cells and thus enhances their cytotoxic potential against bladder tumor cells. This might partially explain the success of BCG in the therapy of superficial bladder cancer. Modulating the expression of adhesion molecules on the tumor target and immune effector cells might improve the efficacy of BCG.

Acknowledgements This work was supported by grant SFB367/C7 from the Deutsche Forschungsgemeinschaft (DFG) to Andreas Böhle.

References

1. Alexandroff A, Jackson AM, O'Donnell M, James K (1999) BCG immunotherapy of bladder cancer: 20 years on. Lancet 353: 1689
2. Axberg I, Ramstedt U, Patarroyo M, Beatty P, Wigzell H (1987) Inhibition of natural killer cell cytotoxicity by a monoclonal antibody directed against adhesion-mediating protein gp 90 (CD 18). Scand J Immunol 26: 547
3. Belldegrun A, Uppenkamp IK, Rosenberg SA (1988) Anti-tumor reactivity of human lymphokine activated killer (LAK) cells against fresh and cultured preparations of renal cell cancer. J Urol 139: 150
4. Berke G, Gabison D, Feldman M (1975) The frequency of effector cells in populations containing cytotoxic T lymphocytes. Eur J Immunol 5: 813
5. Blanchard DK, Hall RE, Dieu JY (1990) Role of CD18 in lymphokine activated killer (LAK) cell-mediated lysis of human monocytes: comparison with other LAK targets. Int J Cancer 45: 312
6. Böhle A, Gerdes J, Ulmer AJ, Hofstetter AG, Flad H-D (1990) Effects of local bacillus Calmette-Guérin therapy in patients with bladder carcinoma on immunocompetent cells of the bladder wall. J Urol 144: 53
7. Böhle A, Nowc C, Ulmer AJ, Musehold J, Gerdes J, Hofstetter AG, Flad H-D (1990) Elevations of cytokines interleukin-1, interleukin-2, and tumor necrosis factor in the urine of patients after intravesical bacillus Calmette-Guérin immunotherapy. J Urol 144: 59
8. Böhle A, Thanhäuser A, Ulmer AJ, Ernst M, Flad H-D, Jocham D (1993) Dissecting the immunobiological effects of bacillus Calmette-Guérin (BCG) in vitro: evidence of a distinct BCG-activated killer (BAK) cell phenomenon. J Urol 150: 1932
9. Brandau S, Böhle A (2001) Activation of NK cells by Bacillus Calmette Guérin (BCG). Eur Urol 39: 518
10. Brandau S, Suttmann H, Flad H-D, Jocham D, Böhle A (2000) Killing of Fas ligand-resistant renal carcinoma cells by interleukin-2- and BCG-activated effector cells. Cancer Immunol Immunother 49: 369
11. Brandau S, Suttmann H, Riemensberger J, Seitzer U, Arnold J, Durek C, Jocham D, Flad H-D, Böhle A (2000) Perforin-mediated lysis of tumor cells by *Mycobacterium bovis Bacillus Calmette-Guérin*-activated killer cells. Clin Cancer Res 6: 3729
12. Brandau S, Riemensberger J, Jacobsen M, Kemp D, Zhao W, Zhao X, Jocham D, Ratliff TL, Böhle A (2001) NK cells are essential for effective BCG-immunotherapy. Int J Cancer 92: 697
13. Bubenik J, Baresova M, Viklicky V, Jakoubkova J, Sainerova H, Donner J (1973) Established cell line of urinary bladder carcinoma (T24) containing tumour-specific antigen. Int J Cancer 11: 765
14. Campbell SC, Tanabe K, Alexander JP, Edinger M, Tubbs RR, Klein EA (1994) Intercellular adhesion molecule-1 expression by bladder cancer cells: functional effects. J Urol 151: 1385
15. de Boer EC, de Jong WH, van der Meijden APM, Steerenberg PA, Witjes F, Vegt PDJ, Debruyne FMJ, Ruitenberg EJ (1991) Presence of activated lymphocytes in the urine of patients after intravesical immunotherapy with bacillus Calmette-Guérin. Cancer Immunol Immunother 33: 411
16. de Landazuri MO, Herberman RB (1972) Specificity of cellular immune reactivity to virus-induced tumours. Nat New Biol 238: 18
17. Donskov F, Basse PH, Hokland M (1996) Expression and function of LFA-1 on A-NK and T-LAK cells: role in tumor target killing and migration into tumor tissue. Nat Immun 97: 134
18. Durek C, Brandau S, Ulmer AJ, Flad H-D, Jocham D, Böhle A (1999) Bacillus-Calmette-Guérin (BCG) and 3D tumors: an in vitro model for the study of adhesion and invasion. J Urol 162: 600
19. El-Demiry MIM, Smith G, Ritchie AWS, James K, Cumming JA, Hargreave TB, Chisholm GD (1987) Local immune responses after intravesical BCG treatment for carcinoma in situ. Br J Urol 60: 543
20. Esavaranathan K, Jackson AM, Alexandrov A, James K (1993) The expression of ICAM-1 & ICAM-2 in bladder cancer cells. J Urol 149: A230
21. Gahmberg CG (1997) Leukocyte adhesion: CD11/CD18 integrins and intercellular adhesion molecules. Curr Opin Cell Biol 9: 643
22. Grimm EA, Rosenberg SA (1984) The human lymphokine-activated killer cell phenomenon. Lymphokines 9: 279
23. Herr HW, Laudone VP, Whitmore WFJr (1987) An overview of intravesical therapy for superficial bladder tumors. J Urol 138: 1363
24. Jackson AM, Alexandrov AB, Prescott S, James K, Chisholm GD (1992) Role of adhesion molecules in lymphokine-activated killer cell killing of bladder cancer cells: further evidence for a third ligand for leucocyte function-associated antigen-1. Immunology 76: 286
25. Jackson AM, Alexandroff AB, McIntyre M, Esuvaranathan K, James K, Chisholm GD (1994) Induction of ICAM 1 expression on bladder tumours by BCG immunotherapy. J Clin Pathol 47: 309
26. Lamm DL, Blumenstein BA, Crawford ED, Montie JE, Scardino P, Grossman HB, Stanisic TH, Smith JA, Sullivan J, Sarosdy MF, Crissman JD, Coltman CA (1991) A randomized trial of intravesical doxorubicin and immunotherapy with bacillus Calmette-Guérin for transitional-cell carcinoma of the bladder. N Engl J Med 325: 1205
27. Larson RS, Springer TA (1990) Structure and function of leukocyte integrins. Immunol Rev 114: 181
28. Lee RK, Spielman J, Zhao DY, Olsen KJ, Podack ER (1996) Perforin, Fas Ligand, and tumor necrosis factor are the major cytotoxic molecules used by lymphokine-activated killer cells. J Immunol 157: 1919
29. Leibold WB, Bridge S (1979) 75Se-release: a short and long term assay system for cellular cytotoxicity. Z Immun Forsch 155: 287
30. Mäenpaa A, Jääskeläinen J, Carpen O, Patarroyo M, Timonen T (1998) Expression of integrins and other adhesion molecules on NK cells: impact of IL-2 on short- and long-term cultures. Int J Cancer 53: 850
31. Morales A, Eidinger D, Bruce AW (1976) Intracavitary bacillus Calmette-Guérin in the treatment of superficial bladder tumors. J Urol 116: 180
32. Ortaldo JR, Mason A, Overton R (1986) Lymphokine activated killer cells: analysis of progenitors and effctors. J Exp Med 164: 1193
33. Ratliff TL, Gillen DP, Catalona WJ (1987) Requirement of a thymus-dependent immune response for BCG- mediated antitumor activity. J Urol 137: 155
34. Ratliff TL, Ritchey JK, Yuan JJJ, Andriole GL, Catalona WJ (1993) T-cell subsets required for intravesical BCG immunotherapy for bladder cancer. J Urol 150: 1018
35. Reiß K, Brandau S, Ulmer AJ, Flad H-D, Jocham D, Böhle A (1999) BCG-immunotherapy of superficial bladder cancer: BCG-induced activation of MNCs is regulated by cytokines. Eur Urol 36: A476
36. Rosenberg SA (1988) Immunotherapy of cancer using interleukin 2: current status and future prospects. Immunol Today 9: 58
37. Sander B, Damm O, Gustafsson B, Andersson U, Hakansson L (1996) Localization of IL-1, IL-2, IL-4, IL-8 and TNF in superficial bladder tumors treated with intravesical Bacillus Calmette-Guérin. J Urol 156: 536
38. Shemtov MM, Cheng DLW, Kong L, Shu W-P, Sassaroli M, Droller MJ, Liu BCS (1995) LAK cell mediated apoptosis of human bladder cancer cells involves a pH-dependent endonuclease system in the cancer cell: possible mechanism of BCG therapy. J Urol 154: 269
39. Springer TA (1990) Adhesion receptors of the immune system. Nature 346: 425

40. Thanhäuser A, Böhle A, Flad H-D, Ernst M, Mattern T, Ulmer AJ (1993) Induction of bacillus Calmette-Guérin-activated killer cells from human peripheral blood mononuclear cells against human bladder carcinoma cell lines in vitro. Cancer Immunol Immunother 37: 105
41. Thanhäuser A, Böhle A, Schneider B, Reiling N, Mattern T, Ernst M, Flad H-D, Ulmer AJ (1995) The induction of bacillus Calmette-Guérin-activated killer cells requires the presence of monocytes/macrophages and T-helper type 1 cells. Cancer Immunol Immunother 40: 103
42. Timonen T, Helander TS (1997) Natural killer cell-target cell interactions. Curr Opin Cell Biol 9: 667
43. Timonen T, Gahmberg CG, Patarroyo M (1990) Participation of CD11a-c/CD18, CD2 and RGD-binding receptors in endogenous and interleukin-2-stimulated NK activity of CD3-negative large granular lymphocytes. Int J Cancer 46: 1035