Urol Res (1997) 25:31–34

ORIGINAL PAPER

E.C. de Boer · L. Somogyi · G.J.W. de Ruiter
Th.M. de Reijke · K.-H. Kurth · D.H.J. Schamhart

Role of interleukin-8 in onset of the immune response in intravesical BCG therapy for superficial bladder cancer

Received: 4 March 1996 / Accepted: 2 September 1996

Abstract In intravesical therapy for superficial bladder carcinoma urothelial cells may, through the production of cytokines, contribute to the bacillus Calmette-Guérin (BCG)-induced local immunological reaction and associated antitumor efficacy. The aim of this study was to investigate such a role for the neutrophil-attracting cytokine interleukin-8 (IL-8). The appearance of IL-8 in patients' urine after BCG therapy was compared with BCG-induced IL-6 and IL-2 and the stability of IL-8 in urine was tested. Compared to IL-6 and IL-2, a rapid induction of IL-8 was observed, occurring after the first BCG instillation. Urinary IL-8 was highly stable, even after 24 h incubation at 37°C. The IL-8 concentration after the first instillation seemed to be associated with subsequent development of an immune response. Consequently, IL-8 seems an attractive candidate for investigation of its prognostic value for a clinical response to BCG therapy.

Key words Interleukin-8 · Bacillus Calmette-Guérin · Bladder carcinoma · Urine

Introduction

Stimulation of the immune system by bacillus Calmette-Guérin (BCG) seems to be a requirement for antitumor efficacy in topical BCG immunotherapy of superficial bladder carcinoma [9, 11, 12]. Urothelial cells might be actively involved in the BCG-induced local immunological reaction. As suggested previously, a mechanism by which this could be achieved is the secretion of cytokines such as interleukin-6 (IL-6) by urothelial cells in response to BCG exposure [8]. Secretion of IL-6 by urinary tract epithelial cell lines has also been reported after stimulation with *Escherichia coli* [10]. Moreover, the cytokine IL-8, produced by urothelial cells, was induced in *E.coli* urinary tract infection [1]. IL-8 (NAP-1) belongs to the CXC family of chemokines and acts as a powerful chemoattractant for neutrophils [2]. Since a rapid influx of neutrophils after BCG instillation is observed in the urine of bladder cancer patients [5], we considered that IL-8, produced by urothelial cells in response to BCG exposure, could be responsible for the attraction of these neutrophils. Locally attracted and activated neutrophils can produce cytokines and proteolytic enzymes [4, 14], which could possibly result in facilitated bladder wall infiltration by leukocytes and/or BCG and enhancement of the immune response. In this manner IL-8 production by urothelial cells might be important for the subsequent development of an immune response against BCG and associated clinical efficacy.

The aim of this study was to investigate whether IL-8 is an early-induced urinary cytokine involved in the onset of the BCG-induced immune response. The appearance of IL-8 in patients' urine after BCG therapy was compared with that of the cytokines IL-6 and IL-2. In addition, the stability of IL-8 in urine was tested to determine whether the properties of this cytokine in urine make it attractive for further investigation, e.g., its value as a predictor of BCG responsiveness.

Materials and methods

Urine collection and cytokine measurement

Nineteen cases (i.e., BCG instillation courses) were studied in 16 patients treated with BCG for superficial transitional cell carcinoma of the bladder. Of these, 14 patients were treated with a first course of BCG. A BCG instillation course consisted of six con-

E.C. de Boer (✉)
Department of Urology, University of Amsterdam,
Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

G. de Ruiter · Th.M. de Reijke
K.-H. Kurth · D.H.J. Schamhart
Department of Urology, University of Amsterdam,
Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

L. Somogyi
Department of Urology, Pécs University, Medical School,
7621 Pécs, Munkácsy M. u. 2., Hungary

secutive weekly intravesical BCG instillations (Connaught, Oncotice or RIVM strain; approximately 5–10 × 10^8 colony-forming units) in 50 ml 0.9% saline after complete transurethral resection of papillary tumor(s). For all patients urine was collected prior to, 2–4 h and 4–6 h after BCG instillation; for six of these patients 8–12 h and 12–24 h samples were also collected. Samples were immediately frozen to −20°C. Specimens were then thawed to a maximum of 4°C, centrifuged to remove cells and debris and stored in aliquots at −20°C until analysis. Since we were primarily interested in the involvement of IL-8 during the initial part of the BCG treatment course, the presence of IL-8 was investigated in urine samples collected during the first 3 weekly BCG instillations. IL-8 was measured in 4 times diluted urine samples using a commercially available IL-8 enzyme-linked immunosorbent assay (ELISA) [Central Laboratory of the Red Cross Blood Transfusion Services (CLB), Amsterdam, The Netherlands]. Cytokines IL-6 and IL-2 in urine were determined as reported previously [9] with commercially available ELISAs (Medgenix, Fleurus, Belgium). Patients with a positive immune response to BCG were defined as having positive urinary IL-2 and IL-6 levels, i.e., > 0.34 U IL-2/μmol creatinine and > 50.1 pg IL-6/μmol creatinine, respectively [9]. Cytokine data were standardized to urine creatinine [9].

In order to investigate the stability of IL-8 in urine, urine samples from three patients with 2015, 2357 and 34011 pg/ml IL-8 were incubated for 2, 4, 8 and 24 h at 4, 20 and 37°C. The amount of IL-8 left after incubation was expressed as a percentage of the concentration at t = 0 h.

Statistical analysis

Differences in urinary IL-8 levels prior to and after the first BCG instillation were analyzed using the Wilcoxon Matched-Pairs Signed-Ranks Test. For other data analyses the Wilcoxon Rank Sum W-Test was used (SPSS software).

Results

In serially collected urine samples from superficial bladder cancer patients, significantly elevated concentrations of IL-8 were found after intravesical BCG treatment. The levels of IL-8 clearly increased (P = 0.0007) after the first BCG instillation compared to the level prior to the first BCG instillation (Fig. 1). In 2 of the 19 cases studied no increase in IL-8 was found. Approximately a 12 times increase in IL-8 (median value; range 1–844) was observed after the first instillation. Urinary IL-8 concentrations after the first instillation were also higher (P = 0.021) compared to IL-8 levels in urine from patients with bacterial cystitis, whereas there was no significant difference (P = 0.092) between the latter and levels in urine collected prior to the first instillation (Fig. 1). IL-8 levels further increased after BCG instillation at weeks 2 and 3 (see below), resulting in a larger difference with samples collected after bacterial cystitis (P = 0.009 and P = 0.002, respectively).

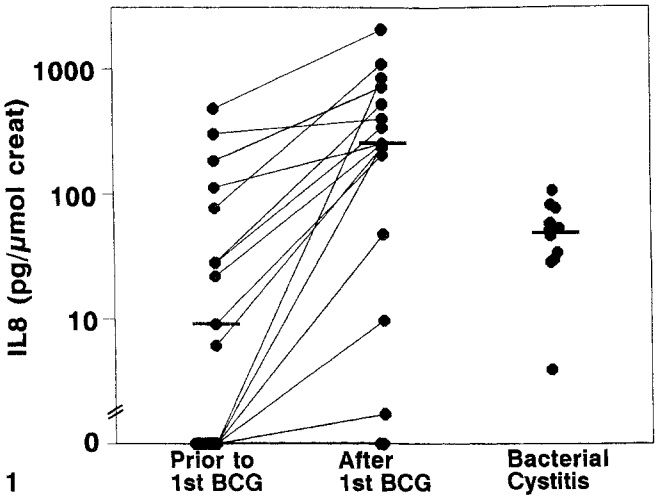
Investigation of the kinetics of IL-8 appearance in the urine during the first 24 h after BCG instillations 1, 2 and 3 revealed that the highest IL-8 concentrations were present at 4 h and 6 h after instillation (Fig. 2). Next the appearance of IL-8 during the first three BCG instillation weeks was studied and compared to IL-6 and IL-2. This was evaluated only for patients treated with a first course of BCG instillations since we previously observed an accelerated appearance of IL-2 during a second or third BCG course (De Reijke et al., manuscript in preparation). The concentration of urinary IL-8 appeared to be clearly elevated after the first BCG instillation, followed by a further increase after instillation weeks 2 and 3 (Fig. 3). IL-8 was the first appearing cytokine during the initial 3 weeks of BCG treatment in comparison with IL-6 and IL-2 in the same patient group.

BCG-induced cytokines such as IFNγ, TNFα, IL-2, IL-6 and IL-1β were previously found to be more or less unstable in the urine [9]. For urinary IL-8 it has been reported that this cytokine was stable at 4 and 20°C [13]. For three IL-8-positive urine samples (t = 4 h or t = 6 h) we investigated the stability of IL-8 by incubating at 4, 20 and 37°C up to 24 h. The results presented in Fig. 4 show that IL-8 was stable in urine even during prolonged (24 h) incubation.

The stability in urine made IL-8 an interesting candidate as a predictor of BCG responsiveness, especially since it is detectable very early during the BCG treatment course. Therefore we investigated whether there was a relationship between early production of IL-8 and subsequent development of an immune response to BCG. Since during an immune response a number of cytokines are produced, the characteristic of having developed an immune response was defined as being positive for urinary IL-2 and IL-6, as described previously [9]. Figure 5 shows that high IL-8 levels after the first BCG instillation were associated with subsequent development of a BCG-induced immune response (P = 0.039). Moreover, IL-8 concentrations after the first instillation were significantly correlated with IL-2 concentrations achieved subsequently during the 6-week course (P = 0.0001, R = 0.767; data not shown).

Discussion

This study showed that IL-8 is a cytokine induced by intravesical BCG application that appears very early during a BCG instillation course compared to IL-6 and IL-2. Urinary IL-8 was highly stable and seemed to be associated with development of an immune response. These characteristics make IL-8 a candidate prognostic factor for clinical response to BCG therapy. Significantly elevated levels of IL-8 were observed after the first BCG instillation, and IL-8 peaked in samples collected at 2–4 h and at 4–6 h. The rapidity of this IL-8 response indicated that a local cell type such as the urothelial cell might be responsible for the secretion of IL-8 after exposure to BCG. This assumption is supported by our observation that IL-8 production by the T24 bladder cancer cell line can be significantly upregulated by incubation with BCG [7]. Furthermore, urothelial cells have been reported to be a major source of IL-8 after deliberate urinary tract colonization with *E. coli* [1]. However, besides urothelial cells IL-8 can be produced by a wide variety of other cells including monocytes, lymphocytes, endothelial cells, neutrophils and pulmonary epithelial cells [2]. Our data do not ex-



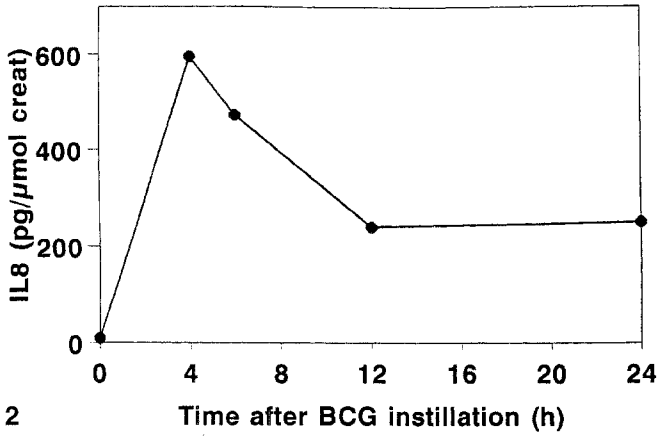
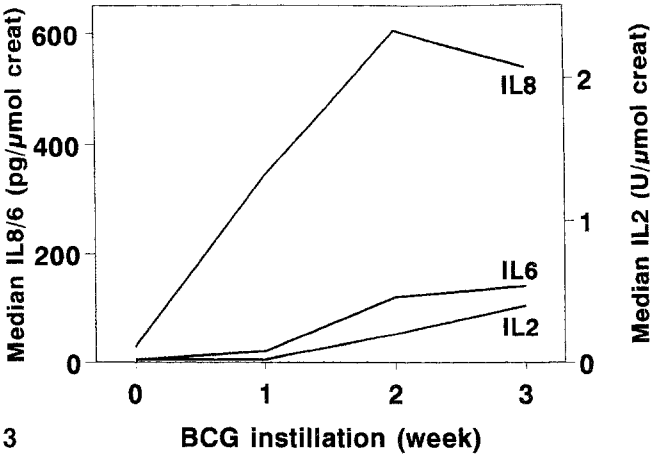


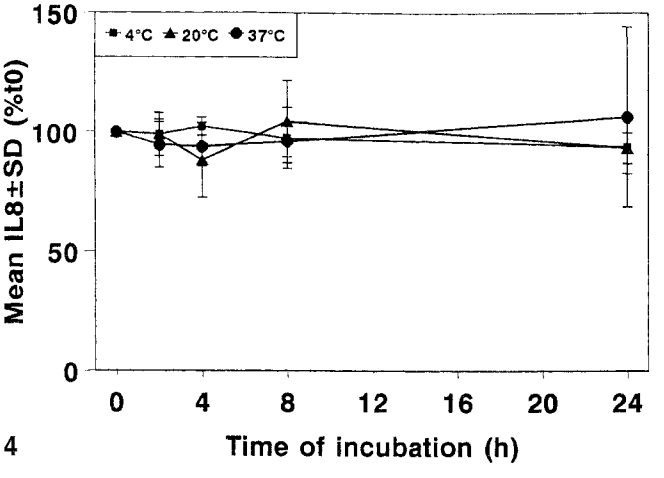
Fig. 1 Levels of IL-8 in the urine from patients with bladder cancer collected prior to and after their first BCG instillation (n = 19) and from patients with bacterial cystitis (n = 11). Urine samples after the first BCG instillation were collected serially; the value of the urine sample with the highest IL-8 concentration is depicted. IL-8 concentrations after the first BCG instillation were significantly higher than prior to the instillation (P = 0.0007). IL-8 levels in urine from bacterial cystitis patients were significantly lower than after the first BCG instillation (P = 0.021) and not different from prior to the first BCG instillation (P = 0.092). *Horizontal bars* indicate median values

Fig. 2 Appearance of IL-8 in the urine during the first 24 h after BCG instillation. Urine was serially collected during the first 24 h after BCG instillation weeks 1, 2 and 3. The median IL-8 level of six patients is shown

Fig. 3 Appearance of IL-8 during the first three BCG instillation weeks compared to IL-6 and IL-2. Urine was serially collected during the first 24 h after BCG instillation weeks 1, 2 and 3 (first course only). IL-2- and IL-6-negative patients were excluded. For each patient the value of the urine sample with the highest IL-8 concentration after each instillation week was evaluated. Median IL-8, IL-6 and IL-2 levels of 11 patients are shown

Fig. 4 Stability of IL-8 in urine at different temperatures. Three IL-8-positive urine samples (t = 4 h or t = 6 h) from different patients were incubated for 0 h, 2 h, 4 h, 8 h and 24 h at 4, 20 and 37°C, after which IL-8 concentrations were determined





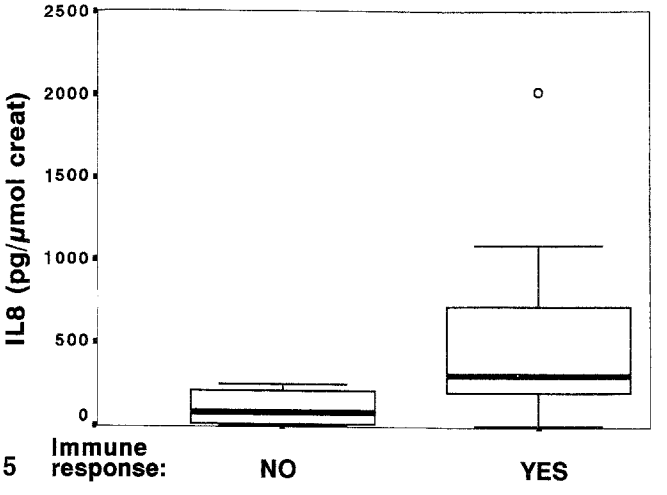


Fig. 5 Box-whisker plots of IL-8 levels after the first BCG instillation in patients with or without immune response. Patients in the group with an immune response (n = 14) were defined as becoming positive for IL-2 and IL-6 in the urine during the BCG therapy course while the other group (n = 5) did not. For individual patients in both groups of serial urine samples the sample with the highest IL-8 concentration was evaluated. The box-whisker plot as used here displays the median and two measures of spread, namely the range and interquartile range. One outlying value is *designated with a circle*. IL-8 concentrations in patients with an immune response were significantly higher than in patients without an immune response (P = 0.039)

clude production of IL-8 by cell types other than urothelial cells.

Although it could have been expected that the urine samples obtained from patients with urinary tract infections contained higher IL-8 levels than samples collected prior to BCG treatment, since patients should be free of urinary tract infection on initiation of BCG therapy, these groups were not significantly different (Fig. 1). The explanation is probably that IL-8 was present in the urine from the bladder cancer patients as a result of previous transurethral tumor resection, as reported by Bettex-Galland and coworkers [3]. Nevertheless, after instillation with BCG a further increase in IL-8 was observed up to a level that was also significantly higher compared to the level in urinary tract infection.

The induction of high IL-8 titers in urine after BCG instillations has been reported previously [3]. However, it was not known whether the IL-8 secretion is involved in the process of intravesical tumor control after BCG instillation, or should be considered solely as a phenomenon associated with inflammation not participating in eradication of cancer cells. Our present data suggest that early IL-8 induction is important for subsequent development of an immune response and thus may be, indirectly, involved in the antitumor efficacy of BCG therapy. Possibly IL-8 acts by attracting neutrophils to the bladder, after which these activated cells facilitate BCG invasion and/or leukocyte infiltration by proteolytic enzyme production [14]. In addition, apart from the traditional involvement in inflammatory response, these neutropils could significantly influence the immune and antitumor response by the release of cytokines [4]. Besides the possible "BCG-induced" permeability, increased permeability of tumors themselves could be of importance too [6].

In conclusion, as cytokine producers urothelial cells may play a more active role in the bladder immune system than was previously recognized. As a consequence the first interaction between BCG and urothelial cells would be an important initializing step in the mode of action of intravesical BCG therapy. The fact that IL-8 is detectable very early during the 6-week induction course of BCG instillations and its stability in urine make it an attractive candidate for prognostic purposes. However, the predictive value of urinary IL-8 for tumor recurrence remains to be established.

References

1. Agace WW, Hedges SR, Ceska M, Svanborg C (1993) Interleukin-8 and the neutrophil response to mucosal gram-negative infection. J Clin Invest 92:780
2. Baggiolini M, Walz A, Kunkel SL (1989) Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 84:1045
3. Bettex-Galland M, Studer UE, Walz A, Dewald B, Baggiolini M (1991) Neutrophil-activating peptide-1/interleukin-8 detection in human urine during acute bladder inflammation caused by transurethral resection of superficial cancer and bacillus Calmette-Guérin administration. Eur Urol 19:171
4. Cassatella MA (1995) The production of cytokines by polymorphonuclear neutrophils. Immunol Today 16:21
5. De Boer EC, De Jong WH, Van Der Meijden APM, Steerenberg PA, Witjes F, Vegt PDJ, Debruyne FMJ, Ruitenberg EJ (1991) Leukocytes in the urine after intravesical BCG treatment for superficial bladder cancer: A flow cytofluorometric analysis. Urol Res 1:45
6. De Boer EC, De Reijke TM, Schamhart DHJ, Vos PCN, Kurth K-H (1993) Increased urinary albumin indicating urothelial leakage following intravesical bacillus Calmette-Guérin therapy for superficial bladder cancer. Urol Res 21:423
7. De Boer EC, Schamhart DHJ, Somogyi L, Kurth K-H (1995) IL-8 and onset of BCG-induced immune response. Urol Res 23:250 (P22)
8. De Reijke ThM, Vos PCN, De Boer EC, Bevers RFM, De Muinck Keizer WH, Kurth K-H, Schamhart DHJ (1993) Cytokine production by the human bladder carcinoma cell line T24 in the presence of bacillus Calmette-Guérin (BCG). Urol Res 21:349
9. De Reijke ThM, De Boer EC, Kurth K-H, Schamhart DHJ (1996) Urinary cytokines during intravesical bacillus Calmette-Guérin therapy for superficial bladder cancer: processing, stability and prognostic value. J Urol 155:477
10. Hedges S, Svensson M, Svanborg C (1992) Interleukin-6 response of epithelial cell lines to bacterial stimulation in vitro. Infect Immun 60:1295
11. Jackson AM, James K (1994) Understanding the most successful immunotherapy for cancer. The Immunologist 2:208
12. Ratliff TL (1989) Mechanisms of action of intravesical BCG for bladder cancer. Progr Clin Biol Res 310:107
13. Thalmann G, Dewald B, Baggiolini M, Studer U (1993) Kinetics and stability of interleukin-8 (IL-8) in the urine after transurethral resection (TUR-B) and after intravesical instillations with bacillus Calmette-Guérin (BCG) in patients with superficial bladder cancer. J Urol 149:337A
14. Van Der Sloot E, Kuster S, Böhle A, Braun J, Wood WG (1992) Towards an understanding of the mode of action of bacillus Calmette-Guérin therapy in bladder cancer treatment, especially with regard to the role of fibronectin. Eur J Clin Chem Clin Biochem 30:503