

ORIGINAL ARTICLE

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Presence of IgE antibodies to bovine serum albumin in a patient developing anaphylaxis after vaccination with human peptide-pulsed dendritic cells

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Abstract Dendritic cells are professional antigen-presenting cells that can be generated in vitro either from monocytes or from CD34⁺ peripheral blood progenitor cells by using recombinant cytokines. These cells have potential implications for immunotherapeutic approaches in the treatment of cancer and other diseases. We have conducted a phase I study in melanoma patients using peptide-pulsed dendritic cells cultured in medium supplemented with 10% fetal calf serum (FCS) and a cocktail of cytokines. Peptide-pulsed dendritic cells were injected intravenously at 2-week intervals. Here we report on a case of type I hypersensitivity anaphylactic reaction after repetitive vaccination with autologous peptide-pulsed cells. Pre-vaccination and post-vaccination serum samples were evaluated for the presence of antibodies to FCS and bovine serum albumin (BSA). A retrospective study in 7 patients vaccinated with FCS-cultured dendritic cells demonstrated the presence of IgG and IgM antibodies to FCS and BSA after vaccination in 6 out of 7 patients. However, IgE antibodies were absent in all patients with the exception of the patient developing anaphylaxis. The patient's serum was demonstrated to contain a strong IgE response directed against BSA. In contrast, 2 patients vaccinated with dendritic cells cultured under

serum-free conditions developed no antibodies to FCS and BSA after repetitive vaccination. We suggest that patients can be sensitized with an IgE response against BSA leading to anaphylactic reactions. On the basis of these data, dendritic cells cultured in autologous serum or under serum-free conditions are recommended for therapeutic applications in vivo.

Key words Dendritic cells · Tumor immunotherapy · Vaccination · Anaphylactic reaction

Introduction

Dendritic cells (DC) are potent antigen-presenting cells and thus specifically involved in the initiation of antigen-specific immune responses [1]. Owing to potent costimulatory activity, they are well-suited to priming naive T cells towards various antigens such as viral peptides or alloantigens. The availability of large numbers of DC that can be generated from hematopoietic progenitor cells or monocytes in vitro has profoundly changed preclinical research and allowed the clinical evaluation of these cells [7, 14, 19]. DC are attractive for in vivo induction and activation of antigen- and peptide-specific T cells. Accordingly, appropriately loaded or transfected DC may be used for vaccination in the field of infectious diseases or in the setting of tumor therapy in order to induce specific cytotoxic T lymphocytes (CTL). The in vitro generation of DC requires the use of cytokines such as interleukin-4 (IL-4)/granulocyte/macrophage-colony-stimulating factor (GM-CSF) and serum or albumin. Several studies using DC in patients have been reported. The cells were cultured either in fetal calf serum (FCS) [13, 16, 18], autologous serum/plasma [3, 8, 9], or in serum-free medium [10]. The route of administration was either i.v., s.c. or direct injection into lymph nodes.

In a recent clinical trial in melanoma patients, autologous peptide-pulsed DC, generated in vitro from CD34⁺ peripheral blood progenitor cells (PBPC) in

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medium containing FCS, were administered i.v. at 2-week intervals [15, 16]. Out of 16 patients, 1 developed an anaphylactic reaction after repetitive vaccination with vasodilatation, leading to drop of blood pressure and constriction of the airways. The reaction was directed against a xenogeneic protein, bovine serum albumin (BSA), contained in the FCS that was used in the culture medium for the *in vitro* generation of DC. Anaphylactic reactions to FCS in patients treated with syngeneic lymphocytes have been previously reported [20]. We now report a non-IgG/IgM-mediated reaction to FCS, consistent with a type I hypersensitivity reaction. To minimize an antibody response to foreign proteins, either an appropriate serum-free medium or autologous serum should be used during the *ex vivo* culture period.

Materials and methods

Patients

Sixteen patients with advanced metastatic melanoma who were HLA-A1- or HLA-A2-positive, were studied in a phase I clinical trial with autologous peptide-pulsed DC [16]. The trial was designed and conducted in accordance with the Declaration of Helsinki. The therapy protocol was approved by the institutional ethics committee and registered with the regulatory state authority as well as the federal Paul-Ehrlich Institute. Written informed patient consent was obtained before entry to the study.

Media and reagents

Cells were cultured in RPMI-1640 medium supplemented with 200 mM L-glutamine, 50 μ M 2-mercapthoethanol, 100 mM sodium pyruvate, 50 μ g/ml streptomycin, 50 U/ml penicillin, minimal essential medium vitamins, and 10% FCS (56 °C for 0.5 h; PAN Systems GmbH, Aidenbach, Germany) (standard culture medium). The following recombinant human cytokines were used at a specific activity of 100 U/ml IL-3 (purity >98%), 100 U/ml IL-4 (purity >98%), 1.6×10^4 U/ml IL-6 (purity >85%), 900 U/ml tumor necrosis factor α (TNF α ; purity >98%), and 0.5 U/ml stem cell factor (purity >98%) (all from CellGenix GmbH, Freiburg, Germany), and 1×10^3 U/ml GM-CSF (purity >95%) (Essex Pharma, Novartis Pharma, Basel, Switzerland).

Mobilization of PBPC and CD34⁺ selection

To mobilize PBPC, patients received daily subcutaneous injections of G-CSF (10 μ g/kg body weight/day; Neupogen, Amgen, Munich, Germany) for 4 days. The PBPC were harvested by leukapheresis and collected from the apheresis product by centrifugation on Ficoll-Hypaque (Pharmacia, Freiburg, Germany) to isolate mononuclear cells. The CD34⁺ progenitor cells were positively selected using the Ceparate avidin-biotin immunoadsorption column (CellPro Inc., Bothell, Wash.).

Generation of DC from CD34⁺ PBPC

DC cells were propagated as described previously, with minor modifications [7]. In brief, cells were cultured for 7 days in standard culture medium supplemented with IL-3, IL-6, and SCF. Subsequently, cells were transferred to standard culture medium supplemented with IL-4/GM-CSF and cultured for the next 3 weeks while the medium was replenished weekly. Cells were seeded at a concentration of 5×10^4 cells/ml at the beginning of culture, and at a concentration of 5×10^5 cells/ml on days 7, 14, and 21. Matu-

ration to interdigitating DC was induced for 24 h by adding TNF α to the culture medium including IL-4/GM-CSF. Between 5×10^6 and 5×10^7 CD1a⁺ DC were pulsed either with HLA-A1-restricted peptides derived from MAGE-1 and MAGE-3 or HLA-A2-restricted peptides derived from Melan-A, gp100, and tyrosinase, selected on the basis of the patient HLA haplotype. DC were pulsed at a final peptide concentration of 10 μ g/ml for 2 h at 37 °C in phosphate-buffered saline (PBS) and washed twice in PBS.

Treatment schedule

Eligible HLA-A1⁺ or HLA-A2⁺ patients received at least four vaccinations of 5×10^6 – 5×10^7 peptide-pulsed DC suspended in 20 ml PBS. The vaccine was administered intravenously, treatment was repeated at 2-week intervals. Two patients received four vaccinations of a different preparation of DC cultured in serum-free medium (CellGro DC medium; CellGenix, Freiburg, Germany). The serum-free medium is based on a modified stem cell growth medium (CellGenix) and the major protein components are human serum albumin and human transferrin, which has been tested according to the standards for *in vivo* therapeutics.

Detection of IgE specific for BSA in patients' sera

A solid-phase immunoassay consisting of a flexible hydrophilic carrier polymer encased in a capsule was used to detect IgE specific for BSA in patients' sera (Pharmacia & Upjohn Diagnostics, Freiburg, Germany). The technical characteristics of the assay are described elsewhere [2, 12]. Briefly, the test uses a carrier consisting of a CNBr-activated cellulose derivative to which BSA is covalently bound. The BSA-bound IgE is detected by a mixture of radiolabeled (¹²⁵I) polyclonal and monoclonal antibodies directed against the Fc portion of IgE. The assay is calibrated against the WHO standard for IgE and results are expressed quantitatively in kilounits per liter (kU/l) for specific IgE, between 0.35 kU/l and 100 kU/l.

ELISA for IgG and IgM to FCS and BSA

A modified alkaline-phosphatase enzyme-linked immunosorbent assay (ELISA) with a highly specific and sensitive alkaline-phosphatase-coupled anti-IgG and anti-IgM was used to determine IgG and IgM specific to FCS and BSA in patients' sera. Polystyrene flat-bottom microtiter plates were coated overnight at room temperature, either with 100 μ l FCS from the same lot used to culture the patient's DC or with BSA (10%, Boehringer Mannheim, Germany). After plates had been washed with PBS/Tween (Tween 20, 0.05%) three times, 150 μ l patients' sera and control sera were added at a 1:100 dilution in PBS/Tween. The plates were incubated at room temperature for 3 h, washed three times with PBS/Tween, and incubated for 3 h either with a goat anti-(human IgG Fc γ fragment) coupled to alkaline phosphatase (Dianova, Hamburg, Germany) or with a goat anti-(human IgM Fc γ fragment) coupled to alkaline phosphatase (Dianova). The alkaline-phosphatase-coupled anti-IgG and anti-IgM were used at a 1:500 dilution in PBS/Tween. Finally, the plates were incubated for 3–30 min with the substrate (*p*-nitrophenylphosphate, 1 mg/ml; Sigma, Deisenhofen, Germany). The absorbance was measured with a spectrophotometer (MR5000; Dynatech Corp., Burlington, Mass.) at 405 nm wavelength.

Results

Anaphylaxis in a single patient after repetitive vaccination with autologous DC cultured in FCS

A 68-year-old man with metastatic melanoma received four i.v. vaccinations containing 1×10^7 autologous

MAGE1/MAGE3-peptide-pulsed DC cultured in FCS. The DC were injected at 2-week intervals without any immediate or delayed side-effects. The patient responded to vaccination with a complete regression of a subcutaneous metastasis, while another small skin metastasis remained unchanged. Another vaccination cycle with 1×10^7 autologous MAGE1/MAGE3-peptide-pulsed DC was started 6 months later. At the third vaccination the patient noted warmth in his hands and feet immediately after the injection followed by a decrease of blood pressure and constriction of the airways. After injection of diphenhydramine and hydrocortisone, the symptoms disappeared without later sequelae.

Anaphylaxis is induced by IgE antibodies to BSA

Determination of the IgE antibody against BSA, the major protein constituent of FCS, was performed with a solid-phase immunoassay in the sera of the patient with anaphylaxis and in another 8 patients, 6 of them receiving peptide-pulsed DC under the same culture conditions, and 2 of them vaccinated with DC cultured in serum-free medium. With the exception of the patient developing an anaphylactic reaction, no increase in the IgE anti-BSA response was observed (Table 1). As shown in Fig. 1, the patient developing anaphylaxis revealed a slight increase in the IgE anti-BSA response from 0.35 kU/l to 4.1 kU/l after the 4th vaccination. During discontinuation of the therapy, the antibodies to IgE returned to baseline levels. Reexposure to FCS-cultured DC (April 1998) resulted in a dramatic increase in the IgE anti-BSA response peaking 4 weeks after development of anaphylaxis with 30.3 kU/l (see Fig. 1).

Table 1 Development of antibodies to fetal calf serum (FCS) and bovine serum albumin (BSA) after vaccination with dendritic cells (DC) cultured in medium containing FCS. Results were expressed as changes of absorbance (405 nm) (IgG, IgM) and kU/l (IgE) in patients' sera at the last vaccination compared to the pre-vaccination sample (+ 4- to 11-fold increase; - 0- to 3-fold increase)

Patient	No. of vaccinations	IgG FCS	IgG BSA	IgM FCS	IgM BSA	IgE BSA
1	7	+	+	+	+	+
2	8	+	+	-	+	-
3	9	-	-	-	-	-
4	4	+	+	+	+	-
5	12	+	+	+	+	-
6	8	+	+	+	+	-
7	4	+	+	+	+	-
8 ^a	4	-	-	-	-	-
9 ^a	4	-	-	-	-	-

^a Vaccination was performed with peptide-pulsed DC, generated in serum-free medium (CellGro DC medium, CellGenix, Freiburg, Germany)

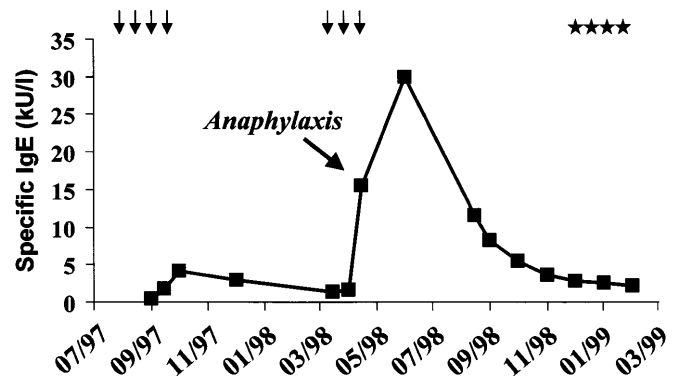


Fig. 1 Induction of IgE antibodies to bovine serum albumin (BSA) in a patient developing a type I hypersensitivity reaction after repetitive vaccination with autologous peptide-pulsed dendritic cells (DC) cultured in fetal calf serum FCS. Determination of IgE antibody against BSA in the patient's serum was performed by solid-phase immunoassay. Results are expressed quantitatively in kilounits per liter (kU/l) for specific IgE. ↓ Vaccination was performed with peptide-pulsed DC cultured in FCS; ★ vaccination was performed with peptide-pulsed DC, generated in serum-free medium (CellGro DC medium, CellGenix, Freiburg, Germany)

Autologous DC cultured in FCS induce IgG and IgM antibodies to BSA and FCS

To evaluate whether anaphylaxis after vaccination with peptide-pulsed DC cultured in FCS is due to IgG and IgM antibodies to BSA or other protein constituents of FCS, ELISA were established with FCS and BSA as the primary coating material. Serum samples from 9 melanoma patients receiving a minimum of four vaccinations with autologous peptide-pulsed DC (7/9 cultured in FCS, 2/9 cultured in serum-free medium) were analyzed for IgG and IgM antibodies to BSA and FCS. As shown in Table 1, 6 out of 7 melanoma patients, vaccinated with DC cultured in FCS, developed IgG and IgM antibodies to FCS and BSA. Figure 2 shows the kinetics of the IgG and IgM response to FCS (Fig. 2A) and BSA (Fig. 2B) after vaccination with peptide-pulsed DC in 1 selected patient. IgG and IgM antibody levels correlated well with the IgE response showing a dramatic increase after reexposure (April 1998), while the total serum IgG and IgM levels remained unaltered (data not shown).

DC cultured in serum-free medium did not cause antibody responses or anaphylactic reactions

None of the patients who received DC cultured in serum-free medium showed signs or symptoms of anaphylactic reactions upon vaccination with peptide-pulsed DC. As shown in Table 1, none of these patients revealed an antibody response against BSA/FCS. The patient who developed a type I hypersensitivity reaction at the 7th vaccination showed no signs of anaphylaxis after four additional vaccinations with autologous peptide-pulsed DC that had been cultured under serum-free conditions. In addition, IgE serum levels remained unaltered during this course of vaccination (Fig. 1).

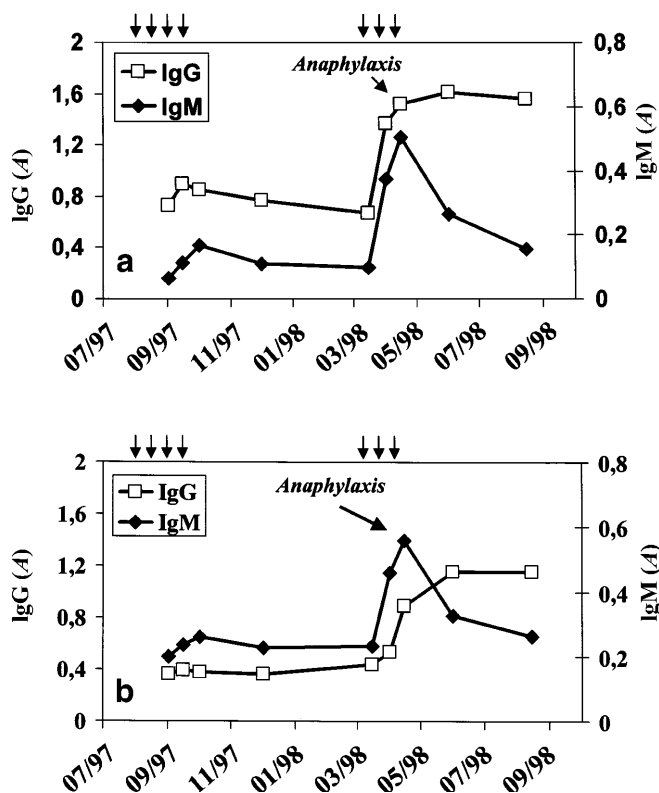


Fig. 2A, B Kinetics of IgG and IgM antibodies to FCS (A) and BSA (B) following repetitive vaccination with autologous peptide-pulsed DC cultured in FCS. Determination of IgG and IgM antibody against FCS and BSA was performed in patients' sera (diluted 1:100) by enzyme-linked immunosorbent assay. Results are expressed as absorbance (A) measured at 405 nm. The absorbance of control sera was less than 0.1 for FCS and less than 0.25 for BSA antibodies. ↓ Vaccination was performed with peptide-pulsed DC cultured in FCS

Discussion

Encouraging preliminary results with approaches involving DC-based tumor vaccination have inaugurated a wave of DC trials [18]. Currently there is no consensus on major issues of DC vaccination, such as DC preparation, loading of DC with tumor antigens, and route of administration. Concerning the DC preparation, the *in vitro* generation from monocytes or CD34⁺ PBPC requires the use of cytokines such as IL-4/GM-CSF and serum or albumin. So far, cells have been cultured either in FCS [13, 16, 18], autologous serum/plasma [3, 8, 9], or in serum-free medium [10]. Here we report on a case of type I hypersensitivity anaphylactic reaction after repetitive vaccination with autologous peptide-pulsed DC cultured in FCS.

Anaphylactic reactions to FCS in patients treated with cultured white blood cells have been previously reported [20]. FCS is frequently used in tissue culture to improve cell viability. In addition, multiple effects of FCS on immune cells have been reported, such as induction of MHC-independent cytotoxicity mediated by T cells [5] and secretion of cytokines [4].

The causative role of FCS in the induction of a type I hypersensitivity reaction in this patient is suggested by (a) the correlation between the development of symptoms and the appearance of antibodies to FCS and BSA and (b) the absence of significant symptoms in the same patient when cells were cultured in serum-free medium instead of FCS. Since 6 out of 7 patients developed IgG and IgM antibodies to BSA and FCS without showing any clinical signs of anaphylaxis, an IgE-mediated reaction to BSA, detected in the patient with the type I hypersensitivity reaction, is suggested to be responsible for the anaphylactic reaction. The clinical symptoms of anaphylaxis in this patient suggest a boosting effect to BSA or other components of the FCS resulting either from previous sensitization by ingestion of beef products or from naturally occurring antibodies [11, 21].

There is an extensive literature on IgE-mediated reactions to xenogenic proteins, mainly from the era of serotherapy (reviewed in [17]). However, the technique of generating recombinant proteins dramatically decreased the incidence of antibody reactions to xenogenic proteins.

The demonstration of IgE, IgG, and IgM antibodies to BSA in our patients after vaccination with peptide-pulsed DC suggests that the washing procedures and final incubation of cells in PBS were inadequate to remove xenogenic proteins from the DC. With the increasing use of *ex vivo* cultured cell preparations like DC, lymphocytes and irradiated tumor cells for immunotherapeutic approaches, it is critical to minimize an immune response to xenogenic proteins, either by appropriate serum-free media or autologous serum/plasma. The use of serum-free medium appears to permit efficient generation of human DC from monocytes and CD34⁺ PBPC [6], without the risk of an antibody response to foreign proteins and anaphylactic reactions.

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