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Peripheral blood dendritic cells and GM-CSF as an adjuvant for hepatitis B vaccination in hemodialysis patients

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Peripheral blood dendritic cells and GM-CSF as an adjuvant for hepatitis B vaccination in hemodialysis patients.

Background. Dysfunctional antigen presentation may underlie the impaired antibody response to hepatitis B vaccination in hemodialysis patients. Dendritic cells are considered to be the most important antigen presenting cells, but their presence and function in hemodialysis patients is unclear. Granulocyte-monocyte-colony stimulating factor (GM-CSF) has been given successfully to hemodialysis patients to increase the proportion of responders to hepatitis B vaccination. Although GM-CSF acts on both monocytes and dendritic cells, the mechanisms underlying its adjuvant quality are largely unknown.

Methods. In this study we analyzed monocytes and dendritic cells in the peripheral blood of hemodialysis patient that had responded to a standard hepatitis B vaccination procedure (responders), patients who had not responded (nonresponders), and healthy controls. The nonresponders were given two additional booster vaccines, both preceded by administration of GM-CSF the day before.

Results. After two booster vaccinations with GM-CSF, six out of seven patients developed a protective antibody response to hepatitis B. The memory T-cell response to tetanus toxoid was significantly lower in nonresponders compared to controls. The monocytes of dialysis patients and healthy controls showed a similar expression of relevant cell surface molecules. However, the numbers of circulating dendritic cells were on average 50% reduced compared to healthy controls, with a further reduction after GM-CSF administration. This was accompanied by a decrease of T-cell proliferation in antigen presentation assays. Monocytes showed increased major histocompatibility complex (MHC) class II, CD54, and CD40 expression, while their antigen-presenting capacity remained unchanged.

Conclusion. GM-CSF is an effective adjuvant for hepatitis B vaccination in primary nonresponding hemodialysis patients, but paradoxically decreases the antigen presenting capacity of peripheral blood mononuclear cells and the number of circulating dendritic cells.

Key words: dendritic cells, dialysis, vaccination, GM-CSF, hepatitis B.

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The impaired immune response after hepatitis B vaccination is a long-standing problem in dialysis patients and is commonly viewed as a symptom of a more generalized impaired immune system in patients with end-stage renal disease (ESRD) [1, 2]. The mechanisms underlying this phenomenon are incompletely understood, although many changes in both immunophenotype and function of monocytes and T cells have been documented in these patients. As early as the late eighties, it was shown that patients that did not respond to a standard hepatitis B vaccination procedure (primary nonresponders) showed a decreased T-cell proliferation after stimulation by monoclonal antibodies directed against CD3. Monocytes of dialysis patients appeared to be relative ineffective antigen presenting cells resulting in decreased interleukin (IL)-2 production by T cells after stimulation. [3]. The T-cell proliferation response could be restored by coculture with transfected fibroblasts expressing the important costimulatory molecule B7, which is normally expressed by antigen presenting cells [4]. Later, Girndt et al [5] reported a small decrease in expression of the B7-2 (CD86) molecule on monocytes of patients on hemodialysis compared to healthy controls. Although peripheral blood T cells from patients with ESRD show signs of activation, they have a normal proliferation capacity after stimulation with cytokines or heterologous monocytes [1, 6, 7]. Based on these findings, the conclusion has been drawn that decreased antigen-presenting capacity of monocytes may underlie the decreased vaccination response in hemodialysis patients.

However, dendritic cells and not monocytes are considered to be the most potent antigen-presenting cell capable of inducing a primary immune response. In the skin they are present as Langerhans cells and dermal dendritic cells that can efficiently take up and process antigen, and thereafter migrate to the draining lymph node for antigen presentation to antigen-specific T cells [8, 9]. This normal physiology of antigen presentation can be very effectively reproduced by subcutaneous vaccination with in vitro antigen-loaded monocyte-derived dendritic cells [10].

Dendritic cells also circulate at low frequency in the peripheral blood before migrating to lymphoid tissues, skin, and organs [9, 11]. They can be differentiated in a myeloid type called DC1 and a lymphoid type DC2, which have different functional characteristics that may result in either preferential T-helper 1 or 2 stimulation [12]. No data are available about the presence and function of circulating dendritic cells in patients with chronic renal failure.

Granulocyte-monocyte-colony-stimulating factor (GM-CSF) is a specific growth factor for hematopoietic progenitor cells and promotes their differentiation into granulocytes, monocytes and dendritic cells. Administration in vivo leads predominantly to granulocytosis and influences phagocytosis, cytotoxicity, and cytokine production of monocytes and granulocytes [13, 14]. GM-CSF is now being recognized as a potential effective adjuvant in many different vaccination procedures [15]. A recent randomized controlled study showed that after an optimal dose of GM-CSF, given before hepatitis B vaccination, all hemodialysis patients developed protective antibody titers [16]. However, the mechanisms underlying the potency of GM-CSF as adjuvant are largely unknown [17].

In the present study we analyzed the phenotype and function of circulating antigen-presenting cells in hemodialysis patients compared to healthy controls, and after administration of GM-CSF.

METHODS

Sixteen patients on chronic intermittent hemodialysis were analyzed. No patient was suffering from a malignancy or autoimmune disease, or was taking immunosuppressive medication. All patients had undergone a hepatitis B vaccination program consisting of four intramuscular injections of 40 μg of hepatitis B vaccine (HB-VAX) (APMSD, Brussels, Belgium) at time 0, 1, 6, and 12 months. Eight patients were considered primary nonresponders as their antibody response to hepatitis B surface antigen (HbsAg) remained below 10 IU/mL (two patients below 2 IU/mL and six patients below 0.1 IU/mL). Normally, no additional boosters are given because in our patient population this does not result in an increased number of vaccination responders. The nonresponders in this study were given two additional intramuscular injections of 40 μg HB-VAX at 1-month intervals in the deltoid region. HB-VAX was always administered on the day of a dialysis session. Twenty-four hours before each of these boosters, GM-CSF (Leucomax; Novartis, Arnhern, The Netherlands) at 3 μg/kg was given subcutaneous. Serum antibody titers for hepatitis B surface antigen were measured before and 1 month after the booster injections.

All patients were in stable clinical condition before and during the study period. They were dialyzed three times weekly for 4 hours. All patients were dialyzed with hemophane (MA-12H) (Kawasumi Laboratory Inc., Tokyo, Japan) dialyzer membranes. Healthy nursing staff and laboratory personnel were the control population.

The local medical ethical committee approved the study and informed consent was obtained from every patient.

Sample preparation

Blood samples were collected in pyrogen-free tubes containing heparin. Blood drawn from hemodialysis patients was always taken before the start of the hemodialysis procedure. On every blood sample a differential cell count was done. Peripheral blood mononuclear cells (PBMC) were isolated layering the blood on a Ficoll-Paque gradient (Amersham Pharmacia Biotech, Uppsala, Sweden). After centrifugation, the PBMC were removed from the interface and washed several times with ice-cold phosphate-buffered saline (PBS) to remove the platelets.

Monocytes were purified from the PBMC by labeling them with anti-CD14–coated magnetic beads. Monocytes were purified by positive selection using MACS CD14 isolation kit (Milteny Biotec, Bergisch, Gladbach, Germany) on an AutoMACS (Milteny Biotec) according to the manufacturer's protocol. This yielded a monocyte population of greater then 98% purity with no detectable dendritic cells present.

Flow cytometric analysis

Whole blood samples were monitored for the expression of cell surface markers on monocytes. Two-color flow cytometry was used after staining with CD14 (phycoerytrin-labeled) as a marker for monocytes in combination with antibodies directly conjugated to fluorescein isothiocyanate (FITC), to detect major histocompatibility complex (MHC) expression (anti-MHC class I and II), adhesion molecules (CD58, CD54, and CD102) and costimulatory molecules (CD40, CD80, and CD86).

Dendritic cells were identified as human leukocyte antigen (HLA)-DR–positive (PerCP-labeled antibody), lineage negative cells (negative for CD3, CD19, CD20, CD16, CD56, CD14, and CD34, all labeled with FITC). DC1 were identified as CD11c (antigen-presenting cells–labeled)-positive and DC2 were identified as CD123 (phycoerytrin-labeled)-positive and CD11c (antigen-presenting cells–labeled)-negative. All antibodies were purchased from Becton Dickinson (Mountain View, CA, USA). The staining procedure was performed by incubating 100 μL of blood with the optimal concentration of antibody for 30 minutes at room temperature, followed

by 10 minutes incubation with fluorescence-activated cell sorter (FACS) lysing solution to lyse red blood cells. Samples were washed twice and flow cytometric analysis was performed on a FACScalibur flow cytometer using Cell Questpro software (Becton Dickinson).

The fluorescence intensity was measured in a semiquantative way to compare the measurements in time [18]. In short, the flowcytometer was calibrated with specific calibration beads in the range of 500 to 50,000 molecular equivalent of soluble fluorochrome (MESF) (Calibration Beads Quantum 1000, RPE medium level) (Bangs Labs Inc., Fishers, IN, USA). The intensity of the fluorescence is converted to a standard curve using Quick Cal program for Quantum Beads (Bangs Lab Inc.). The geomean fluorescence level is denoted as MESF.

In vitro stimulation assays

In all stimulation assays the culture medium was RPMI 1640 (Gibco, Invitrogen Corp., Paisly, Scotland) supplemented with 2 mmol/L glutamine, 100 IU/L penicillin, 100 μg/L streptomycin, and 10% heat-inactivated human AB serum. The allogeneic mixed leukocyte reaction (allo-MLR) was used to test the capacity of PBMC to initiate a primary immune response. Fifty thousand PBMC of the patient were irradiated (30 Gy) and added to 50,000 allogeneic responder PBMC per well of a 96-wells round bottom plate. After 7 days of incubation at 37°C in a humidified atmosphere containing 5% CO_2, [^{3}H]-thymidine (0.5 μCi/well) was added for the last 8 hours of culture. Cells were harvested on a Tomtec 96-well and radioactivity was measured using a 1450 micro-Beta-Trilux counter (Wallac, Turku, Finland). Results were expressed as mean counts per minute. All experiments were done in triplicate.

Allogeneic responder PBMC were harvested from buffy coats obtained from the local blood bank and kept at −140°C until use. Every patient's PBMC sample was tested against PBMC from four different buffy coats. Data are expressed as the mean counts per minute of the four different buffy coats tested.

In a recall antigen proliferation assay the response to nominal antigens like tetanus toxoid and *Candida albicans* was tested. Fifty thousand PBMC in 200 μL of control medium per well of a 96-wells round bottom plate were tested by adding tetanus toxoid (RIVM, Bilthoven, The Netherlands) or *C. albicans* (Artu Biologicals, Lelystad, The Netherlands) in a final concentration of 37.5 limit of flocculation (Lf)/mL and 5 μg/mL. After 7 days of incubation at 37°C in a humidified atmosphere containing 5% CO_2, [^{3}H]-thymidine (0.5 μCi/well) was added for the last 8 hours of culture. Cells were then harvested and radioactivity counted as counts per minute. All experiments were performed in triplicate. As a positive control the proliferation capacity of PBMC was tested with phytohemagglutinin M (PHA-HA16) (Murex Biotech Ltd, Dartford, Kent, UK) at a final concentration of 2 μg/mL added to 50,000 cells per well in a 96-wells round bottom plate.

In all proliferation experiments the PBMC alone showed a background of less than 500 counts per minute.

Statistical analysis

Data obtained at every sample time-point are expressed as means with standard error of the mean. Statistical analysis was performed using the Wilcoxon test for paired observations, analysis of variance (ANOVA), and the Pearson rank correlation test. Results were considered statistically significant when the P value was below 0.05. For multiple comparisons the Bonferroni correction was applied on the P value (0.05/number of groups).

RESULTS

GM-CSF administration and hepatitis B vaccination response

After the first booster of hepatitis B vaccine with GM-CSF, seven out of eight patients developed a measurable antibody titer, but only two patients showed a protective titer of greater then 10 IU/mL. The non-responding patient subsequently did not want to participate in the study anymore. After the second booster six out of seven patients showed a protective antibody titer against hepatitis B surface antigen (average 144 IU/mL, range 11 to 480 IU/mL). The GM-CSF administration was well tolerated and no side effects were reported. However one patient had an asymptomatic rise in body temperature up to 38.8°C (detected by routine body temperature measurement before starting dialysis), the day after GM-CSF administration on both occasions.

Cellular composition of peripheral blood leukocytes

The total number of white blood cells per mL peripheral blood was similar in all groups (Table 1). A relative lymphopenia in hemodialysis patients compared to healthy controls was observed, which has been documented before [1, 18]. The proportions and absolute numbers of dendritic cells were substantially lower in patients on hemodialysis compared to healthy controls. This was observed for both DC1 and DC2 and to a similar degree in both primary responders and nonresponders.

Twenty-four hours after administration of GM-CSF the total number of white blood cells had increased, which was fully accounted for by the increase in number of circulating granulocytes. In all patients the percentage of dendritic cells decreased dramatically resulting in a decrease in total numbers of DC1 and DC2 (Table 1) while the numbers of monocytes remained stable.

Table 1. Differential cell count of peripheral blood leukocytes of healthy controls and hemodialysis patients, which are responders or nonresponders to standard hepatitis B vaccination

	Controls ($N = 8$)	Responders ($N = 8$)	Nonresponders ($N = 8$)	Nonresponders after GM-CSF[a]
Leukocyte count × 10^6/mL	6.4 ± 1.5[b]	4.8 ± 1.5	5.8 ± 1.6	10.7 ± 1.9[c]
Granulocytes %	71.0 ± 7.2	79.0 ± 7.2	73.8 ± 8.8	85.2 ± 5.9[c]
Cell number × 10^6/mL	4.6 ± 1.2	3.8 ± 1.5	4.3 ± 1.3	9.1 ± 1.3[c]
Lymphocytes %	21.7 ± 5.9[d]	15.3 ± 7.1	15.5 ± 8.5	8.3 ± 3.6
Cell number × 10^5/mL	14.3 ± 7.0[d]	6.7 ± 2.4	8.8 ± 5.6	8.9 ± 4.3
Monocytes %	6.3 ± 3.7	6.3 ± 2.6	7.1 ± 4.3	9.6 ± 6.3
Cell number × 10^5/mL	4.3 ± 3.2	2.9 ± 1.3	6.2 ± 4.3	6.4 ± 4.3
Dendritic cells %	0.35 ± 0.96[d]	0.26 ± 0.13	0.20 ± 0.11	0.06 ± 0.02[c]
Cell number × 10^3/mL	23 ± 22[d]	11 ± 5	11 ± 8	7 ± 2.8[c]
DC1				
Cell number × 10^3/mL	15 ± 8[d]	8 ± 4	8 ± 6.6	4 ± 2[c]
DC2				
Cell number × 10^3/mL	6 ± 3.8[d]	3 ± 1.5	3 ± 1.8	2 ± 1[c]

GM-CSF is granulocyte-monocyte-colony-stimulating factor.
[a]Peripheral blood leukocytes obtained 24-hours after subcutaneous administration of GM-CSF to nonresponders. [b]Data are expressed in means with standard error. P values after Bonferroni correction for multiple comparisons were considered significant when below 0.015.
[c]P value <0.01 comparing data from nonresponders before and 24 hours after administration of GM-CSF.
[d]P value <0.01 comparing data from controls with responders and nonresponders.

Cell surface markers of monocytes

The cell surface markers of monocytes are shown in Table 2. No statistical significant differences were observed for the expression of these markers between healthy controls and dialysis patients, nor between responders or nonresponders to standard hepatitis B vaccination (data not shown). Twenty-four hours after administration of GM-CSF major changes were observed. The percentage of MHC class II expressing monocytes increased and was accompanied by an at least fivefold increase in the number of MHC class II molecules per monocyte (expressed semiquantatively as MESF score). Of the adhesion molecules only the cell surface expression of CD54 on monocytes increased significantly. Analysis of the expression of the costimulatory molecules showed that the percentage of CD40-positive monocytes increased (but not the MESF) while the percentage of CD86 bearing monocytes decreased slightly.

Table 2. Expression of cell surface markers on monocytes of primary nonresponders ($N = 8$) before and 24-hours after granulocyte-monocyte-colony-stimulating factor (GM-CSF) administration

	Before GM-CSF	After GM-CSF	P value[a]
MHC class I			
%	100[b]	100	
MESF	29411 ± 15917	23226 ± 14224	NS
MHC class II			
%	83.2 ± 18.8	92.3 ± 6.8	0.05
MESF	7386 ± 5441	38590 ± 14617	0.0001
CD40			
%	35.1 ± 17.8	63.5 ± 15.9	0.0002
MESF	1974 ± 2139	2379 ± 941	NS
CD54			
%	67.3 ± 22.0	91.6 ± 5.5	0.005
MESF	1857 ± 965	5227 ± 2530	0.004
CD58			
%	88.8 ± 23.5	96.8 ± 7.9	NS
MESF	2616 ± 1221	3487 ± 1514	NS
CD80			
%	2.8 ± 1.9	6.1 ± 3.9	NS
MESF	<500	<500	
CD86			
%	99.0 ± 1.9	94.3 ± 4.7	0.03
MESF	8140 ± 3310	4342 ± 3627	NS
CD102			
%	100	100	
MESF	23202 ± 7016	22501 ± 8065	NS

Abbreviations are: MHC, major histocompatibility complex; MESF, molecular equivalent of soluble fluorochrome.
[a]The P values are given for the statistical analysis of the difference in marker expression before and after GM-CSF administration.
[b]Data are expressed as percentage positive cells and MESF in means with standard deviation.

Antigen presentation assays

Proliferation of memory T lymphocytes on recall antigens like tetanus toxoid and *C. albicans* is dependent on effective antigen presentation. Peripheral blood mononuclear cells of the nonresponders in general showed a lower proliferation on *C. albicans* antigen and tetanus toxoid, compared to responders and healthy controls (Fig. 1). In fact, only three out of eight patients in the nonresponders group had a positive response on tetanus toxoid, which was significantly different from healthy controls.

The results of the allo-MLR showed similar proliferation responses in healthy controls and dialysis patients (Fig. 2).

The day after GM-CSF administration, the T-cell proliferation on tetanus toxoid and *C. albicans* antigen decreased substantial in the majority of patients. A similar decrease in proliferation response was observed in the allo-MLR (Fig. 2). Two patients showed an increase in these responses (one patient in both allo-MLR and *Candida* antigen assay and on patient only in the *Candida* antigen assay), but we could not identify a relation with

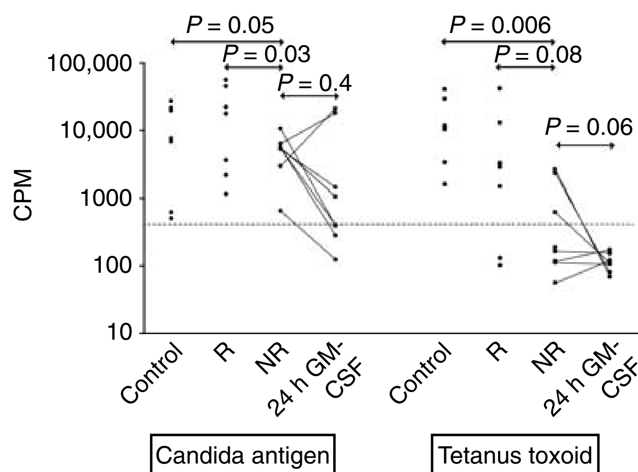

Fig. 1. Proliferation response of peripheral blood mononuclear cells (PBMC) to the recall antigens tetanus toxoid and *Candida albicans*. Data are expressed in counts per minute (CPM). PBMC of eight healthy controls, eight hemodialysis patients who have responded to standard hepatitis B vaccination (R), and eight patients who have not responded (NR) were tested. The PBMC of the nonresponders were tested again 24 hours after subcutaneous administration of granulocyte-monocyte-colony-stimulating factor (GM-CSF). The dotted horizontal line indicates the lower limit of counts per minute above which responses are considered positive (average counts per minute of negative control plus three times the standard deviation). *P* values after Bonferroni correction for multiple comparisons were considered significant when below 0.015.

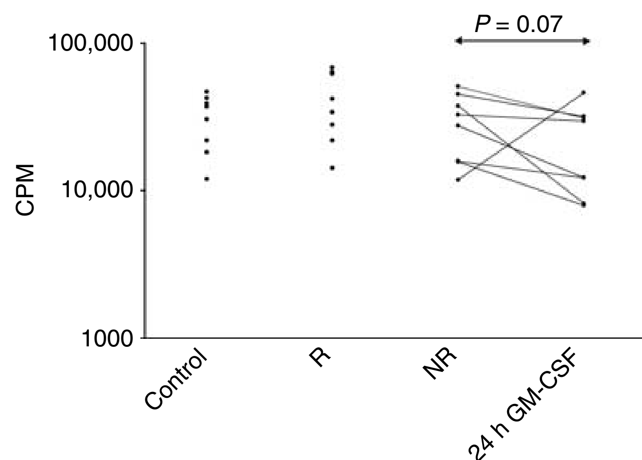

Fig. 2. Proliferative response of allogeneic T cells from buffy coats to peripheral blood mononuclear cells (PBMC). Data are expressed in counts per minute (CPM). PBMC of eight healthy controls, eight hemodialysis patients who have responded to standard hepatitis B vaccination (R), and eight patients who have not responded (NR) were tested. The PBMC of the nonresponders were tested again 24 hours after subcutaneous administration of granulocyte-monocyte-colony-stimulating factor (GM-CSF). Buffy coats or PBMC alone (negative controls) always showed a proliferative response less then 200 counts per minute. *P* values after Bonferroni correction for multiple comparisons were considered significant when below 0.015.

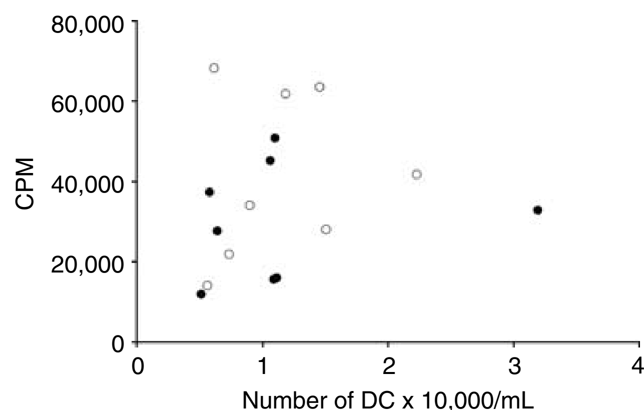

Fig. 3. Relation between number of dendritic cells in the peripheral blood of hemodialysis patients and proliferation response of allogeneic T cells in response to unfractionated peripheral blood mononuclear cells (PBMC) from these patients. Data are shown from eight responder (•) and eight nonresponders (○) to hepatitis B vaccination. Statistical analysis was performed with the Pearson-rank correlation test and showed a *P* value of 0.05. DC is dendritic cells.

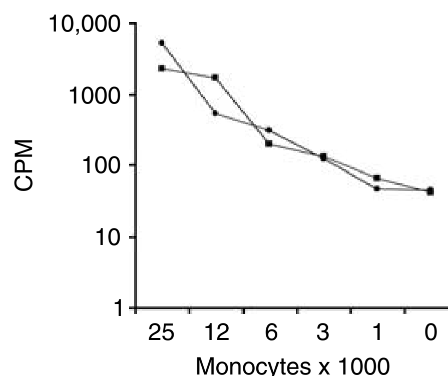

Fig. 4. Proliferation response in counts per minute (CPM) of allogeneic T cells (50,000 cells) to purified monocytes in graded doses before (•) and after granulocyte-monocyte-colony-stimulating factor (GM-GSF) administration (■). Data are expressed as the means of the results obtained with the monocytes from three different hemodialysis patients. The results were similar when the experiment was repeated using allogeneic T cells from a different buffy coat (data not shown).

number of dendritic cells or cell surface expression of costimulatory molecules.

The number of dendritic cells in the peripheral blood of dialysis patients showed a weak but significant relation ($P = 0.05$, $r = 0.42$) with the proliferation responses in the allo-MLR (Fig. 3). Such a relation could not be found for monocytes.

Monocytes from the PMBC were purified to dissect the relative role of monocytes versus dendritic cells as antigen-presenting cells. Of three patients the monocytes were isolated by positive selection on CD14 expression yielding a highly purified population of monocytes with no detectable dendritic cells present. These monocyte populations were tested for their antigen presenting capacity in the allo-MLR using graded numbers of monocytes (Fig. 4). The proliferation response of allogeneic T cells was 10 to 20 times lower with isolated monocytes as antigen-presenting cells then with unfractionated PBMC containing dendritic cells. These responses did not change

when either monocytes before or 24 hours after GM-CSF administration were used.

DISCUSSION

In the present study we used subcutaneous GM-CSF administration as an intervention in the immune system of dialysis patients to potentiate the antibody response to hepatitis B vaccination. In addition the effect of GM-CSF on monocytes and dendritic cells, the two major populations of antigen-presenting cells in the peripheral blood compartment, was analyzed. Confirming the data by others, we showed the efficacy of GM-CSF as an adjuvant for hepatitis B vaccination even in a group of primary nonresponders [16]. The effect of GM-CSF is likely systemic, since it was administered subcutaneously some centimeters from the subsequent intramuscular administered hepatitis B vaccine. The systemic response was also evident since all patients responded with peripheral blood granulocytosis, a decrease in circulating numbers of dendritic cells, and a changed immunophenotype of the monocytes.

There is a paucity of studies documenting the effect of GM-CSF on antigen presentation by monocytes. Recently it was shown that in vitro exposure of human monocytes to GM-CSF for 24 to 48 hours up-regulated the expression of MHC class II, CD40 and marginally CD86 [17]. Prolonged exposure of monocytes to GM-CSF in vitro lead to differentiation into macrophages [19]. Our data are in accordance with the in vitro effects of GM-CSF on monocytes. The day after GM-CSF administration, peripheral blood monocytes of dialysis patients showed both a major increase in MHC class II expression, as well as an increase in the percentage of CD40-positive monocytes. However, unlike the in vitro data, the CD86 cell surface expression on circulating monocytes decreased marginally.

Remarkably, the function of monocytes as stimulators of allogeneic T cells did not change after subcutaneous administration of GM-CSF, despite the major increase in MHC class II expression. Monocytes exposed in vitro to GM-CSF for 24 hours modestly increased their stimulatory capacity for allogeneic T cells, an effect observed only at low monocyte to T-cell ratios [17]. Monocytes are relative poor stimulators of naïve and allogeneic T cells, which at least partly can be contributed to their low expression of co-stimulatory molecules like CD40 and CD86 [8, 20]. In vivo exposure to GM-CSF for 24 hours did not result in a major increase in monocyte surface expression of these molecules, which may explain the unchanged stimulatory capacity in the allo-MLR.

Dendritic cells are the most potent antigen presenting cells in the peripheral blood compartment, especially when the allo-MLR is used as a T-cell proliferation assay. It is assumed that the allo-MLR reflects the capacity of antigen-presenting cells to stimulate proliferation of naive T cells [21]. Especially in vaccination procedures, an induction of the primary immune response, that is switching on the antigen-specific naïve T cells, is the key event that will lead to antibody production by B cells [22]. The percentage of dendritic cells usually does not exceed 1% to 2% of the total cell population in the peripheral blood. However, dendritic cells are at least 30- to 100-fold more potent then monocytes as stimulators of allogeneic T cells. Even at dendritic cells to T-cell ratios as low as 1:900 a significant T-cell proliferation can be monitored [11]. A decrease in numbers of circulating dendritic cells after GM-CSF, as observed in this study, therefore can explain the decrease in proliferation responses in the allo-MLR. This was further supported by the weak, but statistically significant correlation, between numbers of dendritic cells/mL and proliferation of allogeneic T cells. We were unable to find published data on the effect of GM-CSF administration in humans, in relation to dendritic cells. However, one study reported an increased number of circulating dendritic cells after prolonged and high dose administration of GM-CSF in combination with IL-2 [23].

A novel finding was the lower number of circulating dendritic cells in the peripheral blood of dialysis patients compared to healthy controls. We have now extended this finding to patients on peritoneal dialysis and patients with ESRD just before starting renal replacement therapy (manuscript in preparation). Therefore, ESRD per se and not the dialysis procedure, seems to cause this remarkable decrease in circulating dendritic cells. So far the mechanism underlying this decrease in number of circulating dendritic cells is unknown. It may involve a decreased generation of dendritic cells at the level of the bone marrow, or a decreased survival in the peripheral blood. To what extend uremic toxins might influence dendritic cell kinetics and function, is not known.

Two subtypes of dendritic cells have been described, DC1 stimulating Th1 responses and DC2 stimulating Th2 responses. In hemodialysis patients a shift in Th1/Th2 ratio has been described in favor of Th1, which may account for a decreased B-cell response after vaccination [24, 25]. However, we observed a comparable decrease of 50% in both DC1 and DC2 cell count. Despite the decreased number of dendritic cells, the PBMC from dialysis patients and healthy controls stimulated proliferation responses of allogeneic T cells to a similar extent. Because these assays were performed with unfractionated PBMC it may be that other cells like monocytes have influenced the results.

An unexpected and novel finding was the decreased proliferation response of PBMC to recall antigens, specifically tetanus toxoid, in the group of patients defined as primary nonresponders. Based on results from previous studies, it seems likely that this is caused by an impaired

function of antigen-presenting cells [3, 7]. In the study by Gibbons et al, the lowered T-cell responses of hemodialysis patients to the same recall antigens could be restored by MHC class II matched monocytes from healthy controls [7]. A decreased proliferation response of PBMC to PHA has been described before by Meuer et al [3] in dialysis patients not responding to standard hepatitis B vaccination. This response could also be restored by using monocytes from healthy controls. Unlike observations by others, we could not detect a lowered CD86 expression on monocytes of dialysis patients compared to healthy controls [5]. A relative deficiency of CD86 expression on monocytes, with subsequent less co-stimulation via CD28 on T cells, is therefore not the explanation for decreased antigen-presentation capacity in this group of dialysis patients. Monocytes have an antigen-presenting capacity for memory T cells but dendritic cells are far more potent antigen-presenting cells [26]. Therefore, an impaired function of dendritic cells in primary nonresponders may underlie the impaired memory T-cell proliferation responses. In addition, the decrease in dendritic cell numbers after GM-CSF administration may contribute to a further decrease in memory T-cell proliferation responses, as was observed in the majority of patients.

In the current study it was technically not feasible to isolate peripheral blood dendritic cells, because large volumes of blood are required to purify cells that circulate at such low frequency among PBMC. Therefore, we cannot directly document the effect of GM-CSF on dendritic cell function, but could only show the disappearance of these cells from the peripheral blood in conjunction with a decrease in the proliferative responses in the antigen-presentation assays. The monocytes could be highly purified but showed a similar efficacy as stimulator cells for allogeneic T cells before and after administration of GM-CSF. Again it should be emphasized that the stimulatory capacity of monocytes in the allo-MLR is much lower than dendritic cells [11, 21]. To our knowledge this important finding has been neglected in all studies on antigen-presenting cells in patients with ESRD. Even as little as 1% dendritic cells in a monocyte population may greatly affect the results in T-cell proliferation assays.

Dendritic cells leaving the peripheral blood and entering lymphoid tissue and skin after GM-CSF administration, may be the explanation for our paradoxic finding of increased vaccination response and decreased antigen-presenting capacity of PBMC. The increased presence of these pivotal antigen-presenting cells for initiating the primary immune response will lead to the observed adjuvant effect of GM-CSF for hepatitis B vaccination. Direct evidence for this hypothesis is lacking, and only in mice it has been shown that GM-CSF administration increases the number of splenic dendritic cells, especially DC1 [27, 28]. In hemodialysis patients a relative paucity of dendritic cells in the skin has been observed [29, 30]. An influx of dendritic cells from the peripheral blood after GM-CSF administration would certainly benefit the initial phase of the immune response, that is antigen uptake, processing and migration of antigen-loaded dendritic cells to the draining lymph node for presentation tot T cells [8, 9].

Further research is needed to elucidate dendritic cell kinetics and function in uremic patients, in relation to GM-CSF administration and in relation to T-cell responses after vaccination.

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