

Potential involvement of dendritic cells in delayed-type hypersensitivity reactions to β -lactams

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Background: Although the involvement of T cells in delayed reactions to drugs has been studied, little is known about the interaction between the drug and the antigen-presenting cells. Dendritic cells (DCs) are professional antigen-presenting cells essential for initiating T-cell responses. Their ability is regulated in a process known as maturation, by which they modulate the effector immune response.

Objectives: We studied the role of DCs in subjects who had a delayed-type hypersensitivity reaction to amoxicillin to assess the effect on the pattern of maturation and determine the capacity of DCs to activate T lymphocytes.

Methods: We examined the consequences of the interaction between monocyte-derived DCs, lymphocytes, and amoxicillin by means of phenotypic and functional studies, including endocytosis, proliferation, and cytokine production.

Results: Amoxicillin drove DCs from hypersensitive subjects to a phenotypic and functional semimature status, inducing a T-cell proliferation response.

Conclusions: In delayed reactions to amoxicillin, DCs play a relevant role in inducing the T-cell responses. These results are useful not only to understand the mechanism but potentially as a possible approach to diagnosis.

Clinical implications: A better understanding of T-cell and DC involvement in delayed-type hypersensitivity reactions is needed. This *in vitro* assay might provide clues to the diagnostic evaluation of patients allergic to penicillins. (J Allergy Clin Immunol 2006;118:949-56.)

Key words: Delayed-type hypersensitivity, dendritic cells, T cells, cell activation, drug, β -lactam

β -Lactams, the most common agents involved in allergic reactions to drugs, form hapten-carrier conjugates

Abbreviations used

APC:	Antigen-presenting cell
DC:	Dendritic cell
DTH:	Delayed-type hypersensitivity
FITC:	Fluorescein isothiocyanate
imDC:	Immature dendritic cell
LTT:	Lymphocyte transformation test
MFI:	Mean fluorescence intensity
PE:	Phycoerythrin
SI:	Stimulation index

with proteins that can be processed and presented by antigen-presenting cells (APCs) to naive T lymphocytes.^{1,2} In hypersensitive subjects the immune system develops either immediate T_H2-type responses mediated by specific IgE antibodies³ or delayed T_H1-type responses mediated by specific T lymphocytes.⁴ β -Lactams provide the best model to analyze both types of reactions.⁵

Most information about T lymphocyte-mediated reactions to β -lactams concerns the specific effector immune response. *In vitro* studies show that there is a specific lymphocyte proliferation after culture with the culprit drug, and *in vivo* monitoring indicates an increase of CD4 activated cells, CD8 activated cells, or both in both tissue and peripheral blood, parallel to the evolution and the clinical manifestations of the disease.⁶⁻⁸ Little is known, however, about the initial steps mediated by the innate immune system, which is served mainly by dendritic cells (DCs). DCs are professional APCs essential for initiating T-cell responses.⁹ Their abilities are regulated in a process known as maturation, by which they modulate the tolerant or the effector immune responses mediated by different subtypes of T lymphocytes.¹⁰ Although immature DCs (imDCs) and semimature DCs have been related to tolerant immunologic responses and mature DCs to effector responses, this concept seems to be more complex, and other factors must be involved.¹¹ Different studies have supported the role of DCs in the response to haptens.¹²⁻¹⁴ Contact dermatitis, a delayed-type hypersensitivity (DTH) reaction against low-molecular-weight compounds, such as nickel, is the best-studied model.¹⁵ The hapten by itself is able to produce a maturative state in the DCs that induces a specific T-cell response.^{13,15} Apart from these models, we are

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TABLE I. Clinical characteristics and results of the allergologic work-up in the group of patients studied

Patient no.	Sex	Age (y)	Reaction	Time interval (mo)	Skin test (AX)	
					Induration	IHC dermal infiltrate
1	M	37	MPE	196	5 × 6 mm (V)	CD4 ⁺⁺ CD8 ⁺
2	F	50	MPE	171	8 × 6 mm	NT
3	F	60	MPE	180	8 × 10 mm (V)	CD4 ⁺⁺ CD8 ⁺
4	F	52	MPE	60	8 × 8 mm	CD4 ⁺⁺ CD8 ⁺
5	F	43	MPE	66	5 × 7 mm	NT
6	F	43	MPE	132	9 × 9 mm	CD4 ⁺⁺ CD8 ⁺
7	F	57	MPE	31	10 × 11 mm	CD4 ⁺⁺⁺ CD8 ⁺⁺
8	F	49	MPE	55	9 × 11 mm	CD4 ⁺⁺ CD8 ⁺
9	F	54	MPE	240	9 × 7 mm	NT

AX, Amoxicillin; IHC, immunohistochemical (were evaluated as + [10%-25%], ++ [25%-50%], and +++ [more than 50%] of infiltrated marked cells); M, male; MPE, maculopapular exanthema; V, Vesicle formation; F, female; NT, not tested.

unaware of any studies dealing with the involvement of DCs in DTH to drugs.

The aim of this study was to examine the role of DCs in the generation and modulation of the pathologic T cell-mediated DTH response against amoxicillin in patients with homogeneous clinical manifestation (maculopapular exanthema) compared with subjects who tolerated the drug. We reasoned that the immunologic mechanism involved could lie in very early events that include the interactions between the imDCs and the drug. This could modify the maturative status of these imDCs, which could affect the lymphocyte response to the drug. We cultured monocyte-derived DCs *in vitro* with amoxicillin and autologous lymphocytes from hypersensitive patients and control subjects and monitored the phenotypic and functional changes in the imDCs induced by amoxicillin. Subsequently, we studied the proliferative response of the T lymphocytes, changes in their phenotype, and the cytokine secreted. Amoxicillin-stimulated DCs resembled semimature DCs in hypersensitive subjects. These DCs *in vitro* generated the proliferation of a mixed T-lymphocyte population that could correspond to both active and regulatory phenotypes, as occurs when semimature DCs act as APCs. This approach enabled us to demonstrate that stimulation of DCs by amoxicillin could be a triggering factor in the DTH response. Inferring our *in vitro* results to the *in vivo* response, we can speculate that amoxicillin in an adequate environment could not only be the target antigen of the response but also a stimulus of DC maturation. The capacity of DCs to induce proliferation of amoxicillin-specific lymphocytes in hypersensitive patients compared with other APCs, such as B cells or monocytes, could suggest a procedure to improve the *in vitro* diagnosis of the DTH reactions to drugs.

METHODS

Patients and control subjects

Nine patients (mean age, 49.44 ± 7.37 years) with DTH reactions to amoxicillin and a positive delayed intracutaneous test response to amoxicillin were studied.^{16,17} All patients had maculopapular exanthema. Table I shows their clinical characteristics, the time interval between the reaction and sample collection (mean, 156.67 ± 74.91 months), and the results of the allergologic work-up.

Control subjects were composed of 8 healthy sex- and age-matched subjects with no history of hypersensitivity reactions and confirmed good tolerance to penicillins.

The institutional review board approved the study, and informed consent for all the diagnostic procedures was obtained from all patients and control subjects.

Generation and maturation of imDCs

From each patient and control subject, 40 mL of fresh peripheral blood was obtained. Monocytes were purified from the PBMCs by means of plastic adherence by incubating 3 × 10⁶ PBMCs per well in a 12-well plate for 2 hours at 5% CO₂ and 37°C. The nonadherent fraction was washed out thoroughly, placed in aliquots in 10% dimethyl sulfoxide, and frozen. T lymphocytes from this fraction or from PBMCs were separated by using sterile nylon columns. Immature DCs were generated by incubating monocytes for 5 days with 200 ng/mL human recombinant GM-CSF and 100 ng/mL recombinant IL-4 (both from R&D Systems, Minneapolis, Minn), as previously described.⁹

The imDCs were incubated at a concentration of 1 × 10⁵ cells/mL in 48-well plates with amoxicillin (SmithKline Beecham, Madrid, Spain) at 10, 50, or 250 µg/mL, LPS (Sigma, St Louis, Mo) at 1 µg/mL, or TNF-α (R&D Systems) at 10 ng/mL or a combination of amoxicillin plus LPS or amoxicillin plus TNF-α. After stimulation for 24, 48, and 72 hours, culture supernatants were collected and stored at -20°C for subsequent analysis of secreted cytokines. Cells were harvested, and viability was assayed before the next experiments.

Phenotypic analysis of DCs

A total of 2 × 10⁴ treated or nontreated imDCs were incubated for 30 minutes on ice with specific mAbs (CD14-peridinin-chlorophyll-protein complex and HLA-DR-fluorescein isothiocyanate [FITC; BD Pharmingen, San Diego, Calif], CD1a-phycoerythrin [PE], CD80-PE, CD86-FITC, and CD83-PE [Immunotech, Marseille, France]) or with an isotype-matched control. Cells were analyzed by using FACSCalibur flow cytometry, and data were processed with CellQuest (BD Biosciences). Results were expressed as the percentage of positive cells and the mean fluorescence intensity (MFI).

Dextran-FITC endocytosis

Nontreated imDCs or amoxicillin-, LPS-, or TNF-α-treated imDCs (2 × 10⁴) were incubated in 48-well plates with dextran-FITC (molecular weight, 40,000 d; Sigma) at 10 mg/mL for 1 hour at 37°C and at 4°C (nonspecific-binding, negative control). The fluorescence from the internalized dextran was analyzed by means of flow cytometry, and the percentage of positive cells and the MFI were recorded. All data were compared with those for nontreated imDCs at 37°C.

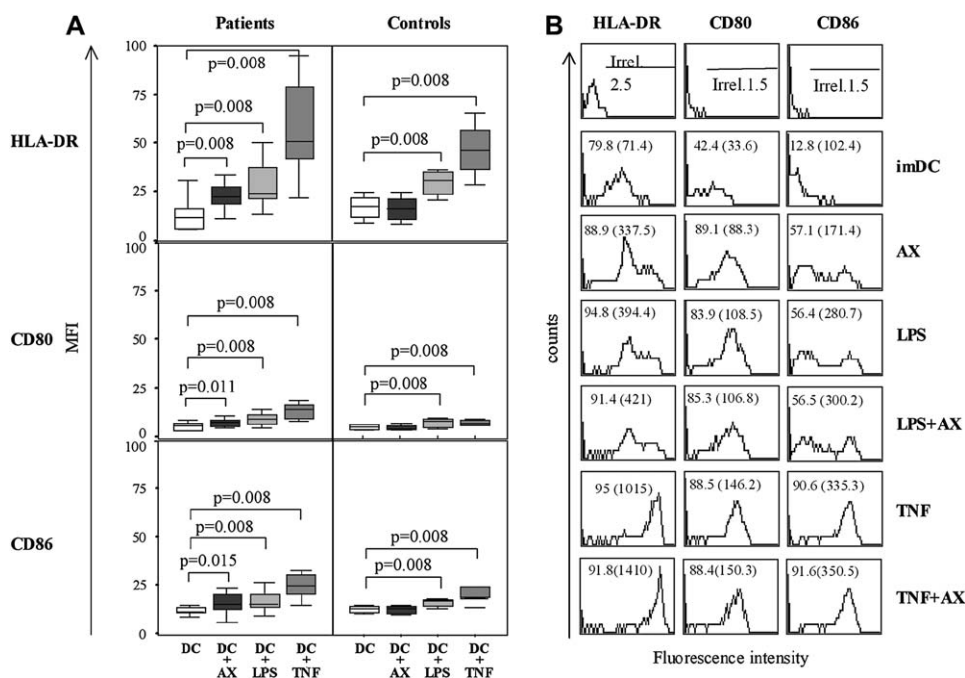


FIG 1. Phenotypic characteristics of DCs. **A**, Phenotypic characteristics expressed as MFI for HLA-DR, CD80, and CD86 before and after culturing with amoxicillin, LPS, or TNF- α in 9 patients (left) and 8 control subjects (right) are shown. **B**, Histograms representative of 3 patients showing phenotype changes after culturing DCs with amoxicillin, LPS, LPS plus amoxicillin, TNF- α , and TNF- α plus amoxicillin are shown. Percentage positivity and MFI are shown in parentheses. AX, Amoxicillin.

Mixed leukocyte reaction

Allogeneic T lymphocytes (1.5×10^5) and 150, 1500, or 15,000 amoxicillin-, LPS-, or TNF-treated and nontreated DCs were cultured into U-bottom 96-well microplates at 37°C in a 5% CO₂ atmosphere. After 4 days, 1 μ Ci of tritiated thymidine (Moravsek Biochemicals, Brea, Calif) was added to each well, and the cells were incubated for an additional 18 hours. The cells were then harvested onto glass fiber filters (Skatron, Tranby, Norway), and the cell-associated radioactivity was assessed in a β scintillation counter (TopCount, Packard Instruments, Downers Grove, Ill). Results were expressed in counts per minute as the mean of triplicate wells.

Activation and proliferation of T cells

The studies were made in parallel by using as APCs either monocytes and B cells from PBMCs (classical lymphocyte transformation test [LTT]) or imDCs. In both experiments autologous T lymphocytes were seeded at 1.5×10^5 cells per well onto U-bottom 96-well microplates. Monocytes and B cells or imDCs previously cultured for 1 hour with amoxicillin at 1, 10, 50, and 250 μ g/mL were added. The lymphocyte/APC ratio was 1:4 for monocytes and B cells and 1:15 for imDCs. Each plate was done in duplicate, one for cell phenotypic analysis and supernatant cytokine determination and the other for proliferative analysis after 6 days' culture. The proliferation was analyzed by using tritiated thymidine incorporation, as described above. Results were expressed as the stimulation index (SI), which was calculated as a ratio of the mean of triplicate counts per minute of each sample by the mean of triplicate counts per minute of lymphocytes alone. An SI of greater than 3 was considered positive.

Lymphocyte phenotypic analysis

Cells were harvested from cultures, and viability was assayed by staining with trypan-blue. For the phenotypic study, 1×10^4 cells were

incubated for 30 minutes on ice with specific mAbs (CD3-peridinin-chlorophyll-protein complex, CD4-APC, CD8-APC, CD25-PE, and HLA-DR-FITC [BD Pharmingen]). Acquisition and analysis were performed as described above for DCs.

Cytokine production

After culture of imDCs with amoxicillin, lymphocytes, or both under different conditions, the supernatants were collected, and the cytokine production (IFN- γ , IL-4, IL-6, IL-10, and IL-12) was analyzed by means of ELISA (Quantikine, R&D Systems, Minneapolis, Minn).

Statistical analysis

Comparisons for quantitative variables between patients and control subjects were done with nonparametric analysis (Mann-Whitney and Kruskal-Wallis tests for nonrelated samples). Comparisons of the results with the different stimuli of these quantitative variables were carried out with the Wilcoxon test for related samples. All reported *P* values represent 2-tailed tests, with values of .05 or less considered statistically significant. The statistical analysis was performed with the SPSS program, version 11.5 (SPSS, Chicago, Ill).

RESULTS

Amoxicillin induced phenotypic and functional changes in imDCs from hypersensitive patients

LPS and TNF- α produced a significant upregulation in the MFI and the percentage of imDCs expressing HLA-DR, CD86, CD80, and CD83 in both patients and control subjects at 24, 48, and 72 hours (Fig 1), with no significant

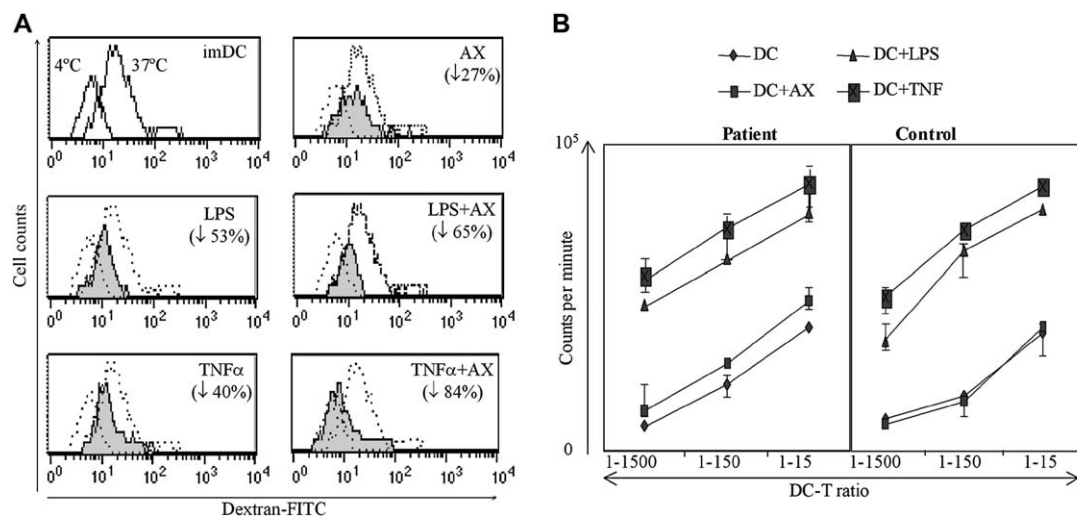


FIG 2. Dextran-FITC intake and allogeneic stimulation in the mixed leukocyte reaction. **A**, DCs from allergic patients were stimulated with amoxicillin, LPS, LPS plus amoxicillin, TNF- α , and TNF- α plus amoxicillin before analyzing dextran-FITC intake. All figures show positive (37°C) and negative (4°C) controls. The percentage of reduction with respect to DCs without stimulus intake at 37°C is shown in parentheses. The experiment shown is representative of 3 with comparable results. **B**, Allogeneic stimulation of T lymphocytes induced by serial numbers of DCs before and after treatment with amoxicillin, LPS, or TNF- α . Each point is the mean counts per minute $\pm$ SD from triplicates of 1 patient (left) and 1 control subject (right). These results are representative of 3 experiments. AX, Amoxicillin.

differences between the 2 groups, indicating that DCs were similar at baseline and responded at equal levels to the maturation stimuli generated by TNF- α or LPS. In hypersensitive patients the results obtained from imDCs stimulated for 72 hours with amoxicillin at 50 and 250 μ g/mL showed significant increases in both the MFI and cell percentages of HLA-DR ($P = .008$ and $P = .012$, respectively), CD86 ($P = .015$ and $P = .008$, respectively), and CD80 ($P = .011$ and $P = .008$, respectively) compared with imDCs without stimulus. No changes in CD83 were detected. The same analysis in control subjects showed no significant changes in any of these markers. The increases induced by LPS and TNF- α were always higher than those induced by amoxicillin (Fig 1, A). In 3 patients tested phenotypic changes in imDCs stimulated with amoxicillin plus LPS and amoxicillin plus TNF- α showed that in all cases the MFI of HLA-DR was upregulated when imDCs were cultured with the combination of amoxicillin plus TNF- α compared with TNF- α alone and with amoxicillin plus LPS compared with LPS alone. No similar changes were observed for CD80 or CD86 (Fig 1, B).

A decrease in dextran-FITC uptake was observed in amoxicillin-treated imDCs compared with in nonstimulated cells. This was greater in LPS- or TNF- α -treated imDCs and even greater with the combination of amoxicillin plus LPS or plus TNF- α (Fig 2, A).

Allogeneic T lymphocytes were added to increasing numbers of imDCs in the absence or presence of amoxicillin, LPS, or TNF- α . After LPS- and TNF- α -induced maturation, DCs from patients and control subjects showed a higher stimulation capacity than in an immature status (Fig 2, B). Compared with untreated DCs, the addition of amoxicillin also resulted in an increased

proliferation of T cells in patients, although not in control subjects. The increase was lower than that generated by LPS or TNF- α (Fig 2, B).

Amoxicillin-loaded DCs are able to present the drug to autologous lymphocytes, inducing proliferation

In only 2 of the 9 patients did lymphocytes proliferate in the presence of amoxicillin using B cells and monocytes as APCs (Fig 3). Nevertheless, when we added imDCs as APCs, a positive proliferation was obtained in 8 of 9 patients (Fig 3). The SI was significantly increased when imDCs were added to the culture ($P = .003$). This effect on the proliferation was only observed in patients and not in control subjects, independently of the APCs added to the culture (Fig 3).

Interaction between DCs, autologous lymphocytes, and amoxicillin induced phenotypic changes in both T lymphocytes and DCs from hypersensitive patients

In 8 of 9 patients, both CD4 and CD8 T lymphocytes proliferated to amoxicillin when DCs were used as APCs, but a clear predominance of CD4 versus CD8 T cells was detected. CD4⁺HLA-DR⁺ and CD8⁺HLA-DR⁺ activated cells were significantly increased ($P = .012$ and $P = .012$, respectively; Fig 4, A). Regarding CD25, a significant increase was observed in CD4 T lymphocytes ($P = .018$) when cultured with DCs and amoxicillin (Fig 4, A). Among total CD4⁺CD25⁺ cells, the dot-plot image showed that a set of CD4⁺CD25^{high} cells was significantly increased ($P = .05$).

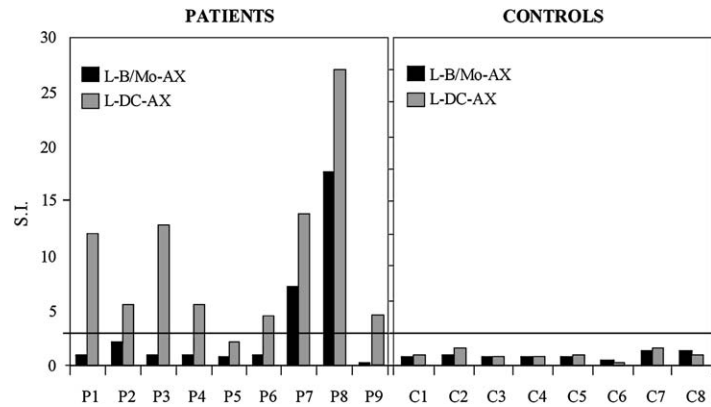


FIG 3. Lymphocyte proliferative response. Proliferative responses against amoxicillin with B cells and monocytes (solid columns) or with DCs (shaded columns) as APCs. The results of 9 patients (left) and 8 control subjects (right) are shown and expressed as the S.I. The solid line shows the cutoff for positive results. AX, Amoxicillin.

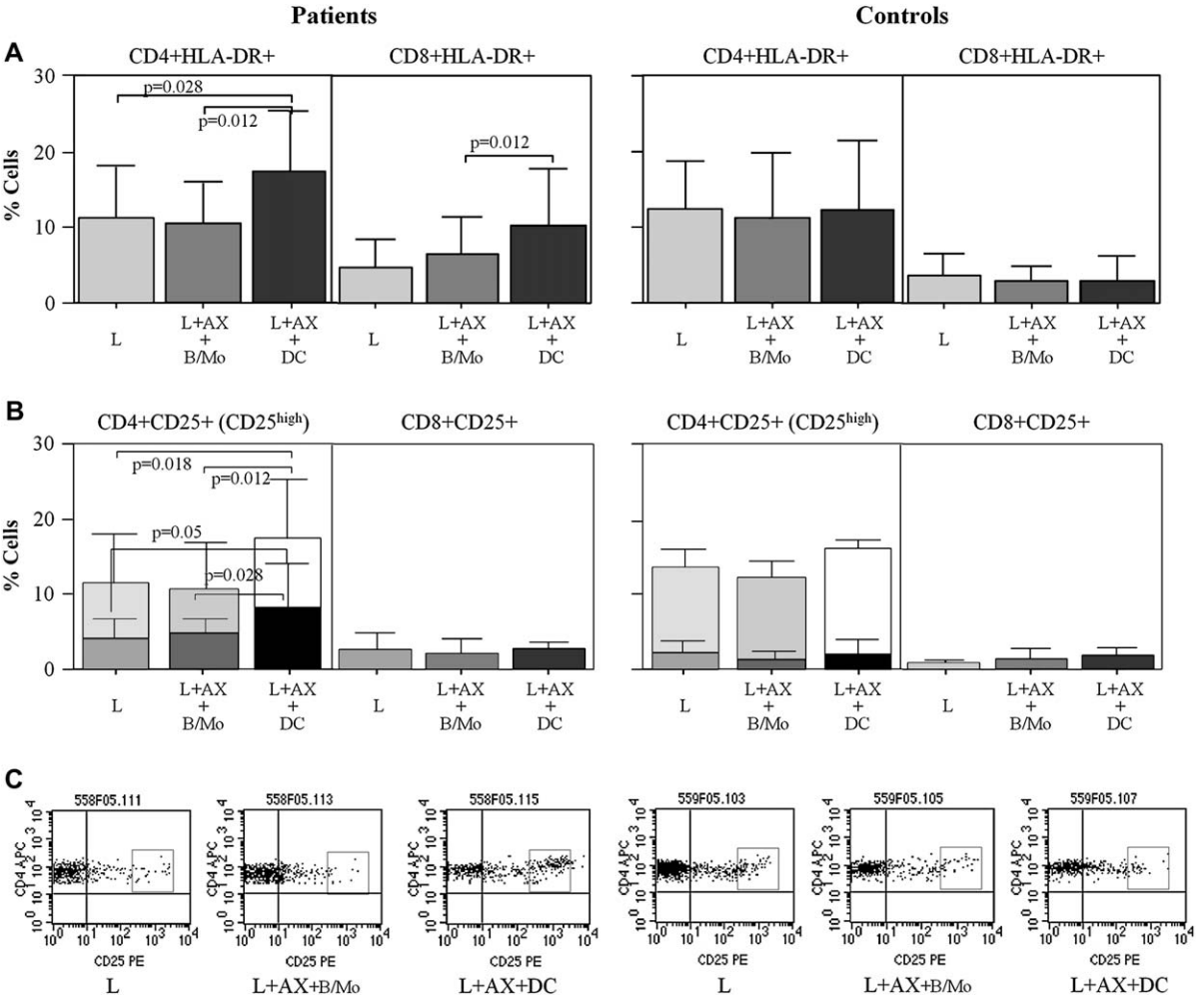


FIG 4. T-lymphocyte phenotype cultured with amoxicillin plus B cells and monocytes or amoxicillin plus DCs (mean percentage $\pm$ SD from 9 patients [left] and 8 control subjects [right]). **A**, CD4⁺HLA-DR⁺ cells and CD8⁺HLA-DR⁺ cells. **B**, CD4⁺CD25⁺ cells and CD8⁺CD25⁺ cells. The subset expressing CD4⁺CD25^{high} is shown in each bar. **C**, Representative dot plots from 1 patient and 1 control subject showing CD4⁺CD25⁺ and CD4⁺CD25^{high} in a frame.

However, in the classic LTT, when B cells and monocytes acted as APCs, no CD4 or CD8 proliferation was detected in 7 of 9 patients, although we detected a significant increase in the percentage of CD8⁺HLA-DR⁺ cells ($P = .02$; Fig 4, A). No changes in the CD25 marker were detected.

Regarding cytokine production, we observed a decrease in IFN- γ in supernatants when T lymphocytes were cultured with amoxicillin and DCs compared with the classic LTT with amoxicillin. We did not detect any production of IL-4. IL-10 values were variable and were not related to the percentage of CD4⁺CD25^{high} T lymphocytes or the proliferation index.

Higher levels of HLA-DR in terms of MFI ($P = .011$) and CD86 in terms of both MFI and percentage of expressing cells ($P = .008$ and $P = .011$, respectively) were obtained when imDCs were cultured with lymphocytes and amoxicillin compared with imDCs cultured only with lymphocytes or only with amoxicillin.

Concerning imDC cytokines (IL-6, IL-10, and IL-12), we detected IL-10 production from nonstimulated imDCs, with a variable response after culture with lymphocytes and amoxicillin: 3 patients showed an increase and 4 showed either a decrease or no change in IL-10 levels. No changes were found in IL-6 levels when compared with imDCs alone or with imDCs plus amoxicillin or lymphocytes. Very low levels of IL-12 production under both baseline and stimulation conditions were observed in just 3 patients: 2 increased and 1 decreased.

No significant changes, either in the lymphocyte or DC phenotype or in cytokine production were detected in the control subjects.

These results showed that in this *in vitro* system with amoxicillin, a bidirectional interaction occurs between DCs and lymphocytes that generates effector and regulatory-type CD4 and CD8 mixed lymphocyte populations that could take part in the *in vivo* response of patients with DTH responses.

DISCUSSION

The aim of this study was to determine whether amoxicillin induces any influence on imDCs in subjects who had a T cell-mediated DTH response. Results showed that amoxicillin increased the expression of DC maturation and activation markers (HLA-DR, CD86, and CD80). Furthermore, amoxicillin-treated imDCs acquired a higher T cell-stimulatory capacity, illustrated by increased proliferation of allogeneic T cells. Also, maturational changes (endocytosis capacity) were noted after amoxicillin-imDC interactions. Although other haptens have been reported to induce DC maturation,^{13,15} to our knowledge, this is the first study showing that a widely used drug like amoxicillin is able to induce maturational changes in imDCs from patients with DTH. However, amoxicillin was unlikely to induce full maturation of DCs because after 72 hours of treatment, no changes were observed in CD83, nor were any changes detected

in the cytokines produced and secreted (IL-10, IL-12, or IL-6). Compared with other agents that interact directly with receptors (LPS and TNF- α), amoxicillin was a less strong stimulator.

Lutz and Schuler¹¹ characterized semimature DCs as those that do not produce proinflammatory cytokines despite the expression of MHC and costimulatory molecules, as occurs with TNF- α -stimulated DCs.¹⁸ Our results with amoxicillin resembled, to a certain extent, this semimature status. Semimature DCs have been proposed as a major component in immune homeostasis.¹⁹ They can induce simultaneously active tolerance against self-antigens derived from peripheral tissues in mice by inducing antigen-specific CD4⁺CD25^{high} (T regulatory cells) or immunity, depending on the type of T cell responding to the presented antigen.¹⁹

Although amoxicillin was not a strong inductor of maturation, what is very exciting is that this effect was detected only in patients with DTH reactions to amoxicillin but not in control subjects. Whether this was due to an intrinsic property of DCs or to factors present in the serum of patients is a matter of speculation. The serum includes cytokines, chemokines, antibodies, apoptotic or necrotic cells, and possibly other factors that can modulate DC maturation.^{9,20} However, no differences were detected between imDCs from patients and control subjects before the addition of amoxicillin or after LPS or TNF- α stimulation. Thus amoxicillin might play a fundamental role in triggering the maturational process. Another possibility could be that amoxicillin generates signals in patients' imDCs, which could induce the release of inflammatory mediators that activate DCs. Aiba et al^{21,22} showed in contact dermatitis that dinitrochlorobenzene induced maturation of DCs, but this was significantly suppressed by either anti-IL-1 β or anti-TNF- α antibodies, suggesting that both proinflammatory cytokines could be involved, whereas the maturation induced by another hapten, NiCl₂, was relatively nonsensitive to treatment with these antibodies, demonstrating differences for each hapten.

In the interactions among DCs, amoxicillin, and lymphocytes, amoxicillin-loaded DCs were able to present this drug to autologous lymphocytes inducing proliferation, being much more potent when compared with other APCs (monocytes and B lymphocytes) used in LTT²³ because monocytes and B lymphocytes were able to induce proliferation in only 2 patients, whereas DCs induced proliferation in 8 of 9 patients. These preliminary results indicate that this approach could be useful for *in vitro* diagnosis. The 2 patients showing proliferation with monocytes and B lymphocytes as APCs were those with the shortest period between the DTH episode and our *in vitro* study, suggesting a higher presence of circulating effector memory lymphocytes,²⁴ which can be stimulated by secondary APCs.²⁵ The time elapsed in the other patients was longer (Table I), and accordingly, these effector cells can be diminished, so that only DCs could act as APCs, interacting with other memory T subsets, probably central memory T cells.²⁶ Another possibility for the different response mediated by DCs regarding monocytes and B cells

could be that DCs induce proliferation of potentially present, specific naive T cells, although 6 days' culture seems to be a short time for this.²⁷ These possibilities need to be verified in further studies.

We determined which lymphocyte subpopulation responses were modulated by the imDCs. There were high proliferation rates, with a predominance of total CD4, but no changes in the CD4/CD8 ratio between the start of culture and 6 days later with amoxicillin-loaded imDCs, which could mean that both subpopulations proliferate. Moreover, activation of both CD4 and CD8 subsets was generated, with an increase in HLA-DR expression, as well as in CD4⁺CD25⁺ cells, when DCs acted as APCs, whereas with monocytes or B cells we detected only slight changes in CD8⁺HLA-DR⁺, as have been reported previously.^{28,29} This finding indicates that in addition to generating a stronger proliferation, DCs induced more complex lymphocyte responses, involving the activation of both CD4 and CD8 subpopulations. With regard to the cytokine production, heterogeneous results were found, as has been described in previous studies.³⁰ This *in vitro* response is similar to *in vivo* data during the reaction, showing that in DTH responses to amoxicillin a mononuclear perivascular infiltrate composed mainly of CD4 cells expresses activation markers and cutaneous homing receptor.^{28,29,31} Regarding the functional significance of these lymphocytes, CD4 T cells regulate CD8 T cell-mediated cutaneous immune responses by restricting the development of effector CD8 T cells.³² This could explain why we detected lower IFN- γ concentrations when DCs acted as APCs compared with monocytes or B cells. This regulatory function might be mediated by the increase in phenotypic-type regulatory (CD4⁺CD25^{high}) T cells detected in our DC-mediated responses.

Regulatory T cells are difficult to phenotype because none of the markers described thus far are expressed exclusively. The most accepted phenotype is CD4⁺CD25^{high}, with FoxP3 positivity.³³ The limitation in cell availability of our *in vitro* system only allowed us to test for an increase in phenotypic CD4⁺CD25^{high}-type cells in the culture but only in the presence of DCs as APCs. Nevertheless, we were not able to find any relationship between the proliferation or IL-10 levels in supernatants with the percentage of CD4⁺CD25^{high} cells. To our knowledge, this is the first study in which DC maturation has been implicated in the induction of T lymphocyte-mediated drug hypersensitivity. The data suggest that amoxicillin seems to be not only the antigen but also a maturative stimulus underlying the pathologic immune response. Amoxicillin by itself in this *in vitro* model was able to enable DCs to generate lymphocyte proliferation of CD4 and CD8 T cells; *in vivo* it is possible that other factors, maybe related with the infectious disease treated with amoxicillin, are needed to induce a clear cytotoxic T_H1 response and the clinical manifestations of exanthema. Because the design of this study involved a large number of cells, not easily available, other determinations were not done. Nevertheless, this is a novel and very interesting approach for the analysis of mechanisms causing

DTH, and that warrants further study³⁴⁻³⁶ to open new insights to explore novel tools for the *in vitro* diagnosis of patients with DTH.

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REFERENCES

- Coleman JW. Protein haptentation by drugs. *Clin Exp Allergy* 1998;28:79-82.
- Weltzien HU, Moulon C, Martin S, Padovan E, Hartmann U, Kohler J. T cell immune responses to haptens: structural models for allergic and autoimmune reactions. *Toxicology* 1996;107:141-51.
- Siraganian RP. Mechanism of IgE-mediated hypersensitivity. In: Middleton E, Reed CE, Elliot EF, Adkinson NF, Yunginger JW, editors. *Allergy: principles and practice*. St Louis: Mosby; 1988. p. 105-34.
- Roujeau JC, Stern RS. Severe cutaneous adverse reactions to drugs. *N Engl J Med* 1994;331:1272-85.
- Schnyder B, Pichler WJ. Skin and laboratory tests in amoxicillin- and penicillin-induced morbilliform skin eruption. *Clin Exp Allergy* 2000;30:590-5.
- Yawalkar N, Egli F, Hari Y, Nievergelt H, Braathen LR, Pichler WJ. Infiltration of cytotoxic T cells in drug-induced cutaneous eruptions. *Clin Exp Allergy* 2000;30:847-55.
- Nassif A, Bensussan A, Boumsell L, Deniaud A, Moslehi H, Wolkenstein P, et al. Toxic epidermal necrolysis: effector cells are drug-specific cytotoxic T cells. *J Allergy Clin Immunol* 2004;114:1209-15.
- Posadas SJ, Padial A, Torres MJ, Mayorga C, Leyva L, Sanchez E, et al. Delayed reactions to drugs show a Th1 profile and levels of perforin, granzyme B and Fas-L related to disease severity. *J Allergy Clin Immunol* 2000;109:155-61.
- Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 1994;179:1109-18.
- Banchereau J, Steinman RM. Dendritic cells and the control of the immunity. *Nature* 1998;392:245-52.
- Lutz MB, Schuler G. Immature, semimature and fully mature dendritic cells: which signals induce tolerance or immunity? *Trends Immunol* 2002;23:445-9.
- Enk AH, Angeloni VL, Udey MC, Katz SI. An essential role for Langerhans cell-derived IL-1 in the initiation of primary immune responses in skin. *J Immunol* 1993;150:3698-704.
- Arrighi JF, Rebsamen M, Rousset F, Kindler V, Hauser C. A critical role for p38 mitogen-activated protein kinase in the maturation of human blood-derived dendritic cells induced by lipopolysaccharide, TNF- α , and contact sensitizers. *J Immunol* 2001;166:3837-45.
- Enk AH, Katz SI. Early molecular events in the induction phase of contact sensitivity. *Proc Natl Acad Sci U S A* 1992;89:1398-402.
- Boislevé F, Kerdine-Romer S, Rougier-Larzat N, Pallardy M. Nickel and DNCB induce CCR7 expression on human dendritic cells through different signalling pathways: role of TNF-alpha and MAPK. *J Invest Dermatol* 2004;123:494-502.
- Romano A, Blanca M, Torres MJ, Bircher A, Aberer W, Brockow K, et al. Diagnosis of nonimmediate reactions to beta-lactam antibiotics. *Allergy* 2004;59:1153-60.
- Barbaud A, Goncalo M, Bruynzeel D, Bircher A. Guidelines for performing skin tests with drugs in the investigation of cutaneous adverse drug reactions. *Contact Dermatitis* 2004;45:321-8.
- Granucci F, Vizzardelli C, Virzi E, Rescigno M, Ricciardi-Castagnoli P. Transcriptional reprogramming of dendritic cells by differentiation stimuli. *Eur J Immunol* 2001;31:2539-46.
- Kleindienst P, Wiethe C, Lutz MB, Brocker T. Simultaneous induction of CD4 T cell tolerance and CD8 T cell immunity by semimature dendritic cells. *J Immunol* 2005;174:3941-7.
- Cella MF, Sallusto F, Lanzavecchia A. Origin, maturation and antigen presenting function of dendritic cells. *Curr Opin Immunol* 1997;9:10-6.

21. Aiba S, Terunuma A, Manome H, Tagami H. Dendritic cells differently respond to haptens and irritants by their production of cytokines and expression of co-stimulatory molecules. *Eur J Immunol* 1997;27:3031-8.
22. Aiba S, Manome H, Yoshino Y, Tagami H. In vitro treatment of human transforming growth factor-beta1-loaded monocyte-derived dendritic cells with haptens can induce the phenotypic and functional changes similar to epidermal Langerhans cells in the initiation phase of allergic contact sensitivity reaction. *Immunology* 2000;101:68-75.
23. Luque I, Leyva L, Torres MJ, Rosal M, Mayorga C, Segura JM, et al. In vitro T-cell responses to β -lactam drugs in immediate and nonimmediate allergic reactions. *Allergy* 2001;56:611-8.
24. Beeler A, Engler O, Gerber BO, Pichler WJ. Long-lasting reactivity and high frequency of drug-specific T cells after severe systemic drug hypersensitivity reactions. *J Allergy Clin Immunol* 2006;117:455-62.
25. von Bergwelt-Baildon MS, Vonderheide RH, Maecker B, Hirano N, Anderson KS, Butler MO, et al. Human primary and memory cytotoxic T lymphocyte responses are efficiently induced by means of CD40-activated B cells as antigen-presenting cells: potential for clinical application. *Blood* 2002;99:3319-25.
26. Lanzavecchia A, Sallusto F. Understanding the generation and function of memory T cell subsets. *Curr Opin Immunol* 2005;17:326-32.
27. Macatonia SE, Hosken NA, Litton M, Vieira P, Hsieh CS, Culpepper JA, et al. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4⁺ T cells. *J Immunol* 1995;154:5071-9.
28. Mauri-Hellweg D, Bettens F, Mauri D, Brander C, Hunziker T, Pichler WJ. Activation of drug-specific CD4⁺ and CD8⁺ T cells in individuals allergic to sulfonamides, phenytoin and carbamazepine. *J Immunol* 1995;155:462-72.
29. Brander C, Mauri-Hellweg D, Bettens F, Rolli H, Goldman M, Pichler WJ. Heterogeneous T cell responses to β -lactam-modified self structures are observed in penicillin-allergic individuals. *J Immunol* 1995;155:2670-8.
30. Hertl M, Merk HF. Lymphocyte activation in cutaneous drug reactions. *J Invest Dermatol* 1995;105(suppl):95S-98S.
31. Torres MJ, Mayorga C, Fernandez TD, Cornejo-Garcia JA, Antunez C, Valenzuela M, et al. T cell assessment in allergic drug reactions during the acute phase according to the time of occurrence. *Int J Immunopathol Pharmacol* 2006;19:119-30.
32. Gorbachev AV, Fairchild RL. CD4⁺ T cells regulate CD8⁺ T cell-mediated cutaneous immune responses by restricting effector T cell development through a Fas ligand-dependent mechanism. *J Immunol* 2004;172:2286-95.
33. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;299:1057-61.
34. Hulette BA, Ryan CA, Gerberick GF. Elucidating changes in surface marker expression of dendritic cells following chemical allergen treatment. *Toxicol Appl Pharmacol* 2002;182:226-33.
35. Ryan CA, Gerberick GF, Gildea LA, Hulette BC, Betts CJ, Cumberbatch M, et al. Interactions of contact allergens with dendritic cells: opportunities and challenges for the development of novel approaches to hazard assessment. *Toxicol Sci* 2005;88:4-11.
36. Rustemeyer T, Preuss M, von Blomberg BM, Das PK, Scheper RJ. Comparison of two in vitro dendritic cell maturation models for screening contact sensitizers using a panel of methacrylates. *Exp Dermatol* 2003;12:682-91.

Correction

With regard to the August 2006 article entitled "Advances in basic and clinical immunology" (2006;118:489-95): In the paragraph under the heading "Regulation of the T-cell response: T_H1 versus T_H2" on page 490, the third sentence should have appeared as follows: "Phosphorylated T bet thus prevented GATA3 from binding its DNA target sites."