

ROLE OF EFFECTOR CELLS (CCR7-CD27⁻) AND EFFECTOR-MEMORY CELLS (CCR7-CD27⁺) IN DRUG-INDUCED MACULOPAPULAR EXANTHEMA

T.D. FERNANDEZ, M.J. TORRES¹, S. LOPEZ, C. ANTUNEZ, E. GOMEZ,
M.F. DEL PRADO³, G. CANTO⁴, M. BLANCA¹ and C. MAYORGA

Research Laboratory for Allergic Diseases, Carlos Haya Hospital-Fundacion IMABIS, Malaga;

¹Allergy Service, Carlos Haya Hospital, Malaga; ²Emergency Service, Carlos Haya Hospital, Malaga; ³Allergy Service, Infanta Leonor Hospital, Madrid, Spain

Received June 5, 2009 – Accepted February 10, 2009

Maculopapular exanthema (MPE) induced by drugs is a T-cell mediated reaction and effector cells may play an important role in its development. We assessed the effector and cutaneous homing phenotype in peripheral blood cells from allergic patients after drug stimulation. This study included 10 patients and 10 controls. The effector phenotype (CCR7-CD27⁺), chemokine receptors (CCR4 and CCR10), and activation (CD25^{low}) and regulatory markers (CD25^{high}) were measured by flow cytometry in both peripheral blood mononuclear cells (PBMCs) and CD4-T-lymphocytes. Proliferation was determined by 5-(6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) assay and the migratory capacity by a chemotaxis assay using CCL17 and CCL27. Compared to controls, CCR7-CD27⁻ cells were increased in patients without ($p=0.003$) and with drug stimulation ($p<0.001$) and had significantly higher proliferation ($p=0.010$). CCR10 expression was increased in patients after drug stimulation in total and memory CD27⁺ T-cells. Lymphocyte migration with CCL27 was higher in patients with drug stimulation ($p=0.048$), with a decrease in CCR7-CD27⁻ ($p<0.0001$) and an increase in CCR7-CD27⁺ ($p=0.017$). In patients, CD4-T-lymphocytes were significantly activated after drug stimulation ($p<0.001$). In conclusion, we show that effector memory CD4⁺ T-cells (CCR7-CD27⁺) respond specifically to the drug responsible for MPE and confirm previous data about the involvement of CCR10 in cell trafficking to the skin.

Non-immediate reactions (NIR) usually occur 24-48h after drug intake, with the skin being the most common target. A variety of clinical entities have been described, with maculopapular exanthema (MPE) as the most frequent manifestation (1). Effector T-cell mediated mechanisms are involved (2), with a predominance of CD4⁺ T-cells found in MPE, with high expression of the cutaneous lymphocyte-associated antigen (CLA) and the

delayed activation marker, HLA-DR (3-4). NIR show a Th1 cytokine pattern, and the production of cytotoxic proteins (perforin and granzyme B) in both skin and peripheral blood (3-6).

Studies in patients with psoriasis and atopic dermatitis have shown that CLA⁺ cells preferentially express the chemokine receptors CCR4 and CCR10, which are involved in cell trafficking to the skin (7-8). Their specific ligands, CCL17

Key words: allergy, skin, t cells, chemokines, cell trafficking

Mailing address: Dr Cristobalina Mayorga Mayorga,
Research Laboratory,
Fundacion IMABIS-Carlos Haya Hospital,
Pl. Hospital Civil, 29009 Malaga, Spain
Tel: ++34 951290224;
Fax: ++34 951290302
e-mail: mayorga.lina@gmail.com

0394-6320 (2010)

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and CCL27, are produced in endothelial cells, keratinocytes, fibroblasts and dendritic cells in this tissue (9). Moreover, CCL27 selectively attracts cells expressing CCR10 in CLA⁺-memory T cells (10). These chemokines and their receptors are also increased in other cutaneous diseases, including NIR to drugs, especially in the most severe reactions, e.g., Stevens-Johnson syndrome (11). We have previously detected, during the acute phase of MPE, the presence CXCL9 and CXCL10 which are Th1 chemokines produced by keratinocytes after IFN- γ stimulation (12) and skin homing chemokines (CCL27 and CCL20) in the affected skin, and their corresponding receptors (CXCR3, CCR10 and CCR6) in both skin and peripheral blood, mainly in CD4⁺ T lymphocytes, indicating the importance of lymphocyte recruitment in this disease (6). In allergic contact dermatitis, the presence of CXCL10 and CXCL9 mRNA has been found in the skin, particularly at sites where lymphocytes are in close contact with, or infiltrated into, the epidermis (12). It has been suggested that macrophages and activated keratinocytes are the main producers of these chemokines that specifically attract the Th1 CD4⁺ lymphocytes to the skin of MPE patients (6).

Although different mechanisms may be involved in lymphocyte trafficking, two main ways are known in the skin, one associated with cutaneous inflammatory processes with CLA⁺CCR4⁺ cells and the other involving CLA⁺CCR10⁺ cells, also showing an effector CD27⁻ and/or CCR7⁻ phenotype (13). Effector T cells are functionally characterized by rapid production of cytokines (IL-4, IFN- γ) and cytotoxic mediators (perforin) (14), and phenotypically by the absence of CCR7 at their surface (14-15), being able to traffic to inflamed tissues using specific receptors (14). Depending on the expression of CCR7, T cells can be divided into central memory (CCR7⁺) or effector memory cells (CCR7⁻) (16). Effector T cells are divided into two subpopulations, depending on the CD27 surface expression: CD27⁺ cells, denominated effector memory T cells (T_{EM}), which are able to respond quickly to antigens (14,15), and CD27⁻ cells, denominated effector cells (T_E), (17) which are more numerous in the skin than the peripheral blood (15). The role of T_E and T_{EM} cells has not been studied in detail in allergic reactions to drugs, although evidence exists of their involvement

and importance in diseases like fixed drug eruption (18). Accordingly, it is tempting to speculate that these cells are those that are mainly implicated in the development of the reaction.

The aim of this study is to analyze the effector (CCR7⁻CD27^{+/+}) and cutaneous homing phenotypes (CCR4 and CCR10) in both peripheral blood mononuclear cells (PBMCs) and CD4⁺ T cells from patients with MPE induced by different drugs, before and after stimulation with the drug responsible for the reaction. We also studied the functional responses by drug-induced proliferation and chemotaxis assays in the presence of two chemokines, CCL17 and CCL27, which are related with skin homing and effector T-cell trafficking. These *in vitro* approaches enabled us to demonstrate the important function of CD27⁺CCR7⁺ and CCR10⁺ T cells in the cellular events in drug hypersensitivity reactions.

MATERIALS AND METHODS

Patients and controls

The study included 10 patients who developed MPE induced by drugs. The allergological work-up followed the European Network for Drug Allergy (ENDA) guidelines (2), and included a clinical history, skin testing (intradermal and/or a patch-test, depending on drug solubility) and, if negative, a drug provocation test (DPT). As controls, we selected 10 sex- and age-matched subjects with good tolerance to the drugs involved in the reactions. No control had any cutaneous or immunological diseases at the time of evaluation. The study was approved by the institutional review board, and informed consent for all procedures was obtained from patients or their parents and controls.

Sample collection

Blood samples were obtained from each patient at least one month after resolution of the reaction. PBMCs were isolated by a Ficoll-Paque density gradient centrifugation (Nycomed, Oslo, Norway) from 20 mL of heparinized venous blood. Serum samples for viral studies were also obtained and stored at -20°C until analysis.

NK removal from peripheral blood mononuclear cells

In order to avoid cytotoxic interference produced by NK cells and to study directly the role of effector T cells, NK cells were removed from PBMCs by positive selection using magnetic beads (Miltenyi Biotech, Bergisch Gladbach, Germany), according to the manufacturer's instructions. The separation efficiency was assessed by

flow cytometry.

Subpopulation phenotype analysis

The determination of different effector T-lymphocyte subsets was performed by flow cytometry, as previously described (19). Briefly, a total of 10^5 PBMCs were incubated with the specific monoclonal antibodies: CD4 (PE, APC, PerCP), CD27-AlexaFluor 647, CCR7-PECy-7, CCR4-PE, CD25-PECy-7 (all provided by Becton-Dickinson, San Jose, CA) and CCR10-PE (R&D Systems, Minneapolis, MN). Following two washes, cells were resuspended in PBS and a six-parameter analysis was performed with Cell Quest software on a FACSCalibur cytometer. Negative isotype controls were used to verify the staining specificity of the antibodies used.

Intracellular cytokine production

Cytokine production by the different T-cell subpopulations was measured following stimulation of PBMCs with ionomycin (1 $\mu\text{g/mL}$) (Sigma, St. Louis, MO) overnight and for the last two hours they were incubated in the presence of brefeldin A (10 $\mu\text{g/mL}$) (Sigma). The PBMCs were then stained with the corresponding monoclonal antibodies for the cell surface markers, and fixed and permeabilized with FIX & PERM cell kits (Caltag Laboratories, Burlingame, CA). After this, the cells were intracellularly stained with PE-labelled IFN- γ , TNF- α and IL-4 monoclonal antibodies (Becton-Dickinson). The acquisition and analysis of the cells were done as described above.

CFSE dilution-proliferation assay

PBMCs without NK cells were incubated at $5\text{--}15 \times 10^6/\text{mL}$ in PBS with 5% foetal calf serum (FCS) (BioWhittaker, Pittsburgh, PA) and a solution of CFSE (5-(and-6)-carboxyfluorescein diacetate succinimidyl ester) (Molecular Probes, Eugene, OR) in DMSO (Sigma) was added to a final concentration of 5 μM at 37°C for 10 min. The reaction was stopped with 1 mL of 20% FCS culture medium at room temperature for 2 min. Cells were immediately washed two times in Stemline T-cell Expansion Medium (STEM) (Sigma) and cultured at 15×10^4 per well, in quadruplicate with the culprit drug, at a final concentration shown in Table I, or with anti-CD3 (Becton-Dickinson) at 1 $\mu\text{g/mL}$ and tetanus toxoid (Calbiochem, Darmstadt, Germany) at 100 $\mu\text{g/mL}$ as positive control. All these concentrations were tested in previous experiments and shown not to be toxic for the cells and to produce a good response in allergic patients (data not shown). Cells were maintained at 37°C in 5% CO_2 for 7 days. As the fluorescence intensity of CFSE-stained cells halves with each cell division, proliferative cells are easily identified and enumerated for the loss of

CFSE content by flow cytometry. The proliferation results were expressed as a proliferation index (PI) calculated as the ratio between percentage of CFSE^{low}+CFSE⁻ cells obtained in cultures with stimulus and those without stimulus.

Migration assay

Transwell chemotaxis assay was performed using a 24-well microchemotaxis chamber with 5 μm pores (Corning Coster Corporation, Cambridge, MA), as described (20). Briefly, the recombinant chemokines, CCL17 and CCL27 (R&D-System Inc, Minneapolis, MN) were diluted in STEM at 100 nM, and 600 μL was placed in the lower chamber. The PBMCs, after CFSE staining and 7 days' culture with the culprit drug, were resuspended in STEM at 1×10^7 cells/mL and 100 μL was placed in the upper chamber. After incubation at 37°C for 4 h in 5% CO_2 , the membrane filters were removed from the chamber and the migrating cells were collected, counted in a Neubauer chamber and stained with the above mentioned markers for cytometric analysis as described previously.

Statistical methods

Results were expressed as the median and interquartile range (IR). Comparisons for quantitative variables without a normal distribution were done by non-parametric analysis, Kruskal-Wallis tests for non-related samples and Wilcoxon test for related samples. All reported p values represent two-tailed tests, with values <0.05 considered statistically significant. The statistical analysis was performed using the SPSS program, version 11.5.

RESULTS

The different parameters were analyzed in the 10 patients finally included in the study, 7 female and 3 male, (mean age, 55.1 years) who developed an MPE confirmed as being induced by drugs. The drugs involved in the reactions and the results of the allergological work-up (skin test and DPT) of the patients are shown in Table II. In all cases a viral infection was ruled out by viral serology.

CCR7-CD27⁻ T and CD4⁺ lymphocytes specifically respond to drug involved in the reaction

The percentage of effector (CCR7-CD27⁻) and effector memory (CCR7-CD27⁺) T cells and CD4⁺ cells in patients and controls was analyzed by flow cytometry with and without stimulation with the culprit drug (Fig. 1). We followed the gating strategy as shown in Fig. 1A by selecting lymphocytes that

Table I. *Drugs and final concentration used for cell cultures.*

Drug	Final Concentration
Amoxicillin (SB Smithkline Beecham, Madrid, Spain)	250 µg/mL
Alopurinol (Faes Pharma, Vizcaya, Spain)	250 µg/mL
Iomeprol (iomeron®, Rovi Laboratory, Madrid, Spain)	250 µg/mL
Clindamicin (Pfizer, Madrid, Spain)	250 µg/mL
Lamotrigin (Lamictal®, Glaxo-Wellcome, Madrid, Spain)	250 µg/mL
Phenytoin	6.25 µg/mL

Table II. *Clinical characteristics and results of the allergological work-up of the patients included in the study.*

Pat.	Sex	Age	Drug	Skin test	DPT
1	Female	72	Amoxicillin	IDT +	n.d.
2	Male	55	Alopurinol	PT/IDT -	+
3	Male	66	Iomeprol	PT/IDT -	+
4	Female	53	Clindamicin	PT/IDT -	+
5	Female	61	Amoxicillin	IDT +	n.d.
6	Female	15	Phenytoin	IDT +	n.d.
7	Female	62	Lamotrigin	IDT +	n.d.
8	Male	68	Iomeprol	PT/IDT -	+
9	Female	34	Phenytoin	PT/IDT -	+
10	Female	65	Iomeprol	PT/IDT -	+

a) IDT: Intradermal test, PT: Patch test, n.d.: not done

were composed of T and B lymphocytes, as the NK cells had already been removed. We avoided any possible interference from the CD4⁺ low-intensity cells proceeding from the monocytes by just selecting

the lymphocytes, using the SSC and FSC parameters. The results showed an increase in the percentage of CCR7⁺CD27⁺ cells in patients compared to controls, both without (p=0.003) and with drug stimulation

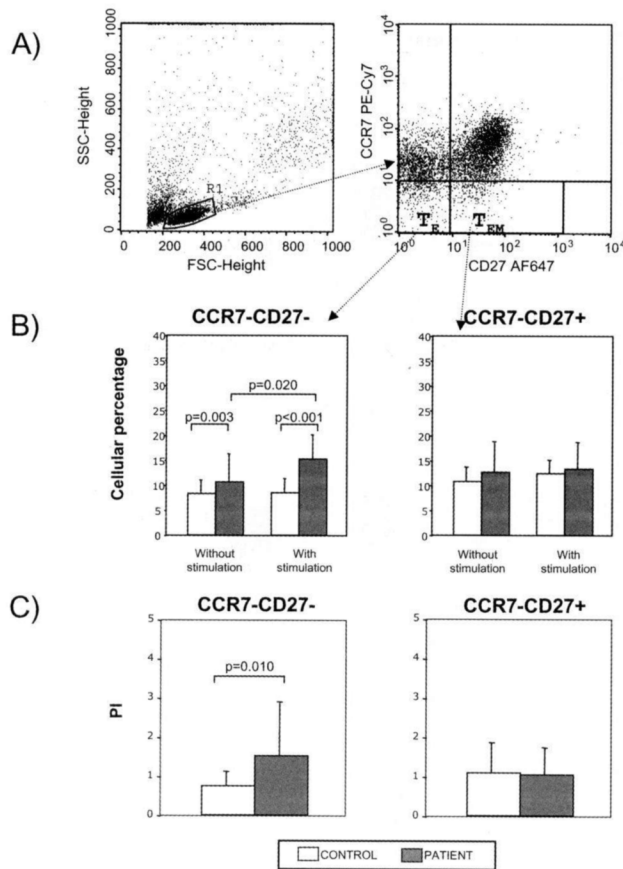


Fig. 1. PBMCs from patients and controls were cultured with and without drug stimulus after elimination of NK cells. Cells were marked with CFSE just before the start of the culture. **A)** Graph showing the gating strategy to analyze the effector T lymphocytes. **B)** Mean of the percentage of T_E cells (CCR7-CD27⁻) and T_{EM} cells (CCR7-CD27⁺) in patients and controls after 7 days of culture, without and with the culprit drug. **C)** Mean PI in T_E and T_{EM} cells in patients and controls. Statistical analysis was done using Kruskal-Wallis tests for non-related samples and Wilcoxon test for related samples.

($p < 0.001$), with no differences in the effector memory cells (Fig. 1B). Analysis of related samples showed that there was also a significant increase in the percentage of CCR7-CD27⁻ cells in patients when PBMCs were stimulated with the culprit drug ($p = 0.020$) (Fig. 1B). The same analysis carried out specifically on CD4 T lymphocytes showed no

differences (Data not shown).

The proliferative response after stimulation with the culprit drug of these two T-cell subsets, effector (CCR7-CD27⁻) and effector memory (CCR7-CD27⁺), was also analyzed in patients and controls. The results showed a significant increase in the proliferating CCR7-CD27⁻ cells in patients compared to controls ($p = 0.010$) (Fig. 1C).

CCR10 expression is increased in T_{EM} and both $CD4^+$ - T_{EM} and $CD4^+$ - T_E after drug stimulation in patients

We assessed the expression of CCR10 in both effector (CD27⁻) and memory (CD27⁺) T cells and in the CD4⁺ T-cell subpopulation in patients and controls, both with and without stimulation by the culprit drug. Following the gating strategy shown in Fig. 2A, the results showed that CCR10 expression without drug stimulation was lower in patients compared to controls in all T-cell subsets analyzed, T cells, T_E and T_{EM} ($p < 0.001$) (Fig. 2B, top). Analysis of the results obtained in allergic patients after drug stimulation showed a significant increase in CCR10 expression in total lymphocytes and T_{EM} ($p = 0.012$ and $p = 0.05$, respectively), but not in the T_E subpopulation (though the values were higher). In contrast, a significant decrease of CCR10 in all T-cell subpopulations was observed in controls ($p = 0.001$, $p = 0.014$ and $p < 0.001$, respectively) (Fig. 2B, top). Stimulation with positive controls, like anti-CD3, also induced a decrease (although not significant) in CCR10 expression in controls similar to that occurring with drugs, whereas no changes were observed in patients (Fig. 2C). Similar results were obtained when the analysis was focused on the CD4 T cells, with an additional significant increase in the T_E subpopulation in patients after drug stimulation ($p = 0.036$) (Fig. 2B, bottom).

Effect of homing and effector chemokines on the migration of different T-cell subpopulations

We analyzed the migration capacity of different T-lymphocyte subpopulations by incubating the cells in a transwell plate with or without CCL17 or CCL27, in the presence or absence of the drugs (Fig. 3). The results showed an increased migration of total lymphocytes only when they were incubated with CCL27 in controls without drugs ($p = 0.031$),

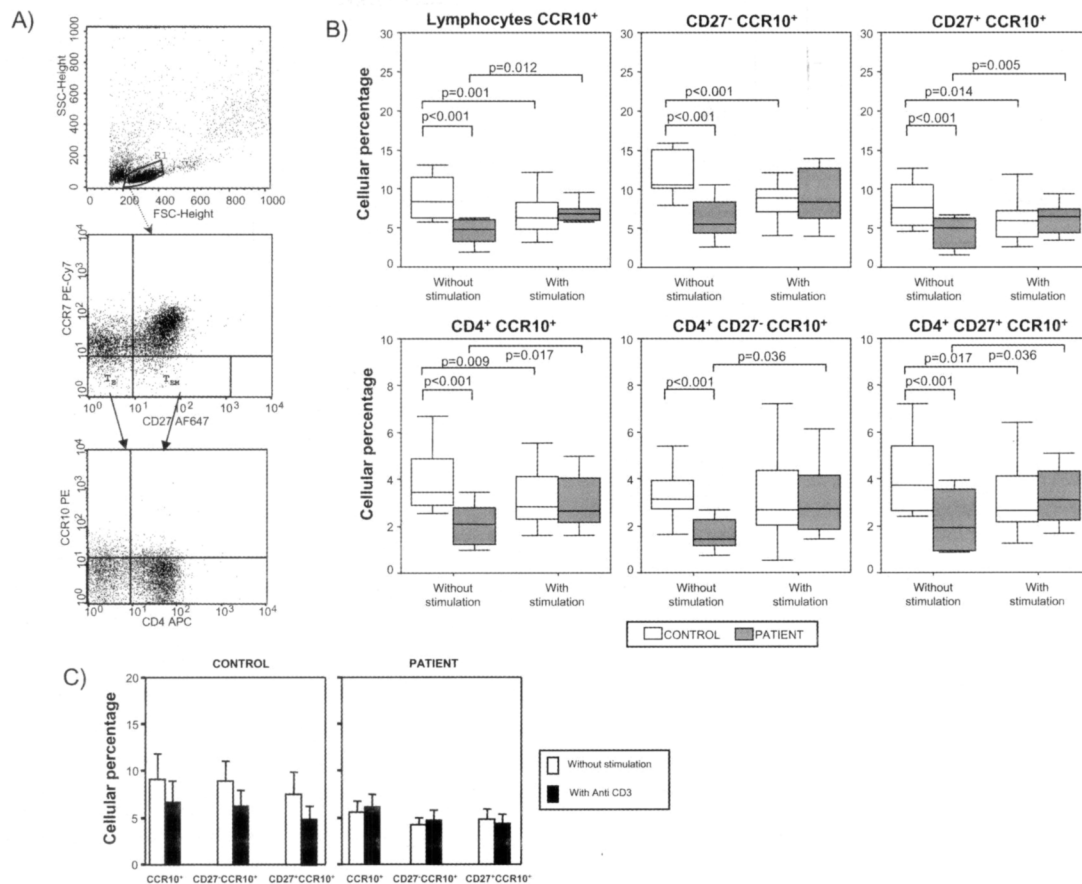


Fig. 2. *A)* Figure showing the gating strategy to analyze the effector T lymphocytes and CD4 lymphocytes expressing CCR10. *B)* Box plots represent the expression of CCR10 in T lymphocytes, T_E cells (CD27⁻), T_{EM} cells (CD27⁺), CD4⁺ T cells, CD4⁺ T_E and CD4⁺ T_{EM} cells in patients and controls after 7 days of culture, without and with culprit drug stimulation. *C)* Comparisons of CCR10 expression in T lymphocytes, T_E cells (CD27⁻), T_{EM} cells (CD27⁺) after 7 days of culture, with or without antiCD3 stimulation, in patients and controls. Statistical analysis was done using Kruskal-Wallis tests for non-related samples and Wilcoxon test for related samples.

and in patients with drug stimulation ($p=0.048$), indicating that in this latter group, stimulation with the drugs is necessary for cell migration in the presence of CCL27 (Fig. 3A). No significant changes in the migration were observed for any of the groups analyzed when incubated with CCL17.

Comparisons of the migrating cell phenotype by a non-parametric test for related samples only showed significant changes in the patients for T_E and T_{EM} (Fig. 3B). Data showed that after drug stimulation there was a decrease in the percentage of migrating T_E cells (CCR7⁻CD27⁻) ($p<0.0001$) and an increase in migrating T_{EM} cells (CCR7⁻CD27⁺) ($p=0.017$) in the

presence of CCL27. CCL17, however, only induced a significant increase in migration in T_{EM} cells from patients after drug stimulation ($p=0.004$) (Fig. 3B).

Cytokine production of different T-cell subpopulations

The intracellular cytokine production (IFN- γ , TNF- α and IL-4), measured by FACS analysis, showed no differences after drug stimulation in the different T-cell subpopulations evaluated (Fig. 4).

Activation and regulatory marker expression

CD25 is a molecule that forms part of the IL-2

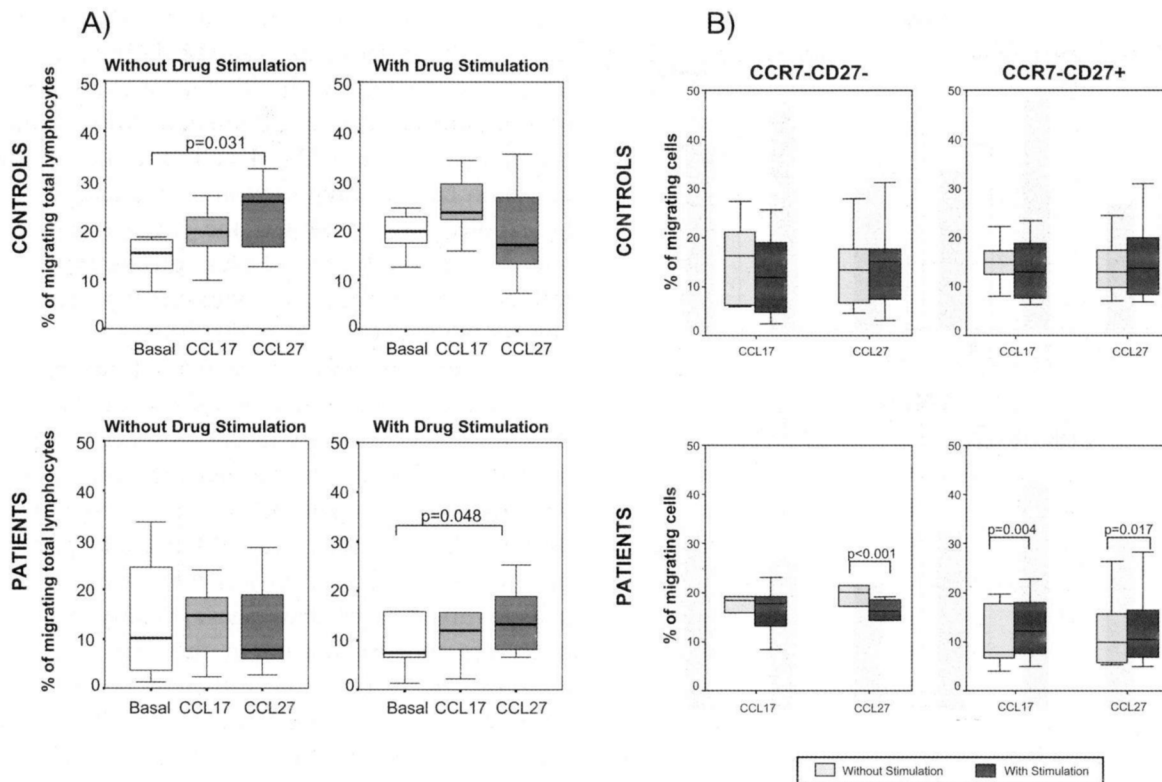


Fig. 3. *A)* Box plots represent the percentage of total lymphocytes that migrate spontaneously, with CCL17 or CCL27, after 7 days of culture, without and with culprit drug stimulation, in patients and controls. *B)* Box plots represent the percentage of T_E cells (CCR7-CD27⁻) and T_{EM} cells (CCR7-CD27⁺) that migrate with CCL17 or CCL27, after 7 days of culture without and with culprit drug stimulation, in patients and controls. Statistical analysis was done using Wilcoxon test for related samples.

receptor. Although it is expressed in CD4 T cells after their activation it has been demonstrated that CD4⁺ cells that express CD25 with high fluorescence intensity in FACs analysis represent the CD4 T regulatory cells in humans (21). We evaluated how incubation with the drug involved in the reaction may influence the CD25 marker with both a low (activation) and a high (regulatory) intensity on CD4⁺T-lymphocytes (Fig. 5). The CD25^{low} expression was increased in patients compared to controls after drug stimulation ($p<0.001$) (Fig. 5A). However, the CD25^{high} expression was significantly lower in allergic patients compared to controls ($p=0.038$) without drug stimulation. This difference disappeared after stimulation with the culprit drug because there was a slight increase in T cells

expressing this marker in allergic patients.

The expression of the homing chemokine receptor, CCR4, only showed a significant difference in CD4⁺CD25^{high}CCR4⁺, lower in patients compared to controls, but, as occurred with CD4⁺CD25^{high}, the difference was only observed before drug stimulation ($p=0.017$) (Fig. 5B).

DISCUSSION

The aim of this study is to characterise the effector cells implicated in exanthematic maculopapular reactions induced by drugs. In previous studies our group has shown that the cells mainly involved at the acute phase of MPE induced by drugs were CD4⁺ with cytotoxic capabilities (perforin and

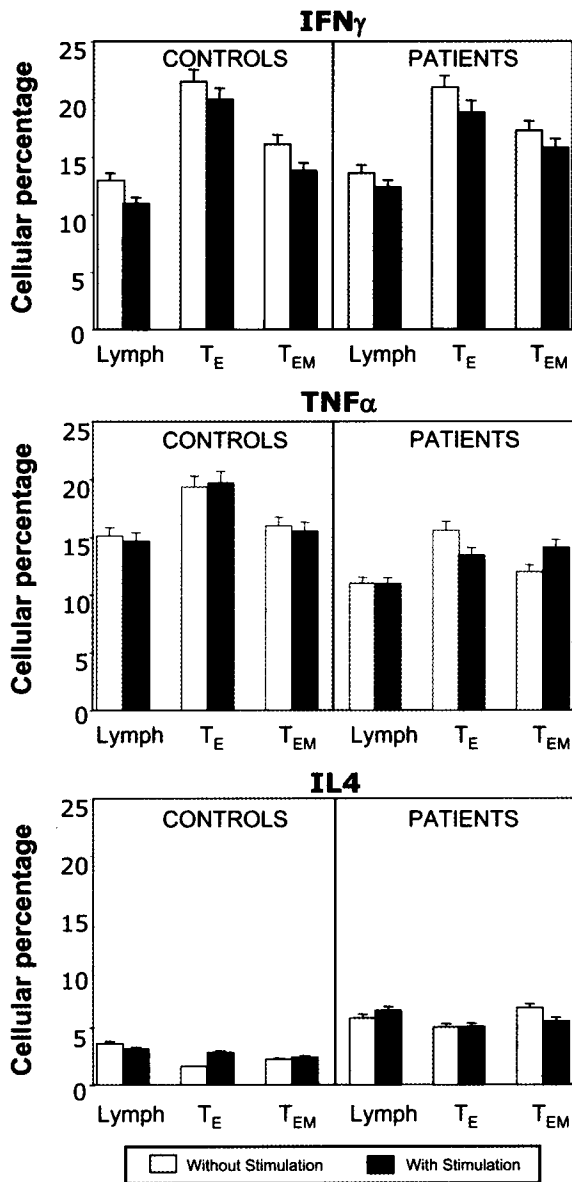


Fig. 4. Mean and standard deviation of the percentage of IFN γ , TNF α and IL-4 production by lymphocytes, T_E cells (CCR7-CD27⁻) and T_{EM} cells (CCR7-CD27⁺) in patients and controls after 7 days of culture, without and with the culprit drug.

granzyme B), with a Th1 phenotype in cytokine production (IFN- γ and TNF- α) and chemokine receptor expression (CXCR3) (6). Moreover, these cells showed a skin homing phenotype with expression of CLA and chemokine receptors (CCR6 and CCR10). All these findings have been observed in the two compartments involved in the allergic

reaction, peripheral blood and the affected tissue (skin) (3, 6), thus demonstrating the importance of Th1 cell trafficking with effector functions between these tissues for the development of MPE. Although the involvement of CD4⁺ T cells and their cytokine secretion has been reported in drug allergy (22-23), no studies have analysed the involvement of T_E and T_{EM} cells in these diseases considering also the influence of chemotactic cytokines and regulatory cells in the development of the disease.

In the present work we carried out further studies by characterizing the effector cell subpopulations and their migratory capacity in the presence of the drug inducing the reaction. We analyzed these effector subpopulations in peripheral blood from patients during the resolution phase of a confirmed drug-induced MPE. This decision was taken since the cells involved in the acute phase, mainly the effector cells, are mostly located in the skin and cannot therefore be detected in sufficient percentages in peripheral blood. Using CD27 and CCR7 markers, four subpopulations can be defined: CCR7⁺CD27⁺ (naïve and central memory cells, T_{CM}), CCR7⁻CD27⁻ (effector cells, T_E), CCR7⁻CD27⁺ (effector-memory cells, T_{EM}) and CCR7⁺CD27⁻, with an unknown function (24,25). In some cutaneous diseases with an immunological basis, such as systemic lupus erythematosus, CD4 T_E and T_{EM} cells were shown to be increased (26). However, information about the role of these cells in allergic reactions to drugs is lacking.

In this study, the percentage of T_E lymphocytes was significantly increased in patients compared to controls. This increase may be because the inflammatory process was still active, given the short time interval between the reaction and the study, even though the symptoms had already resolved. When these cells were stimulated with the culprit drug, the differences persisted and were significantly higher than in the same cell subpopulation without drug stimulation, which may indicate that those antigen-specific cells involved in NIR to drugs are T_E. We also found a specific T_E proliferation to the culprit drug only in patients. The fact that the T_E cells proliferated is in contrast with previous reports showing that effector cells have no proliferative capacity (16, 24). A possible explanation for this proliferation concerns the duration of cell culture

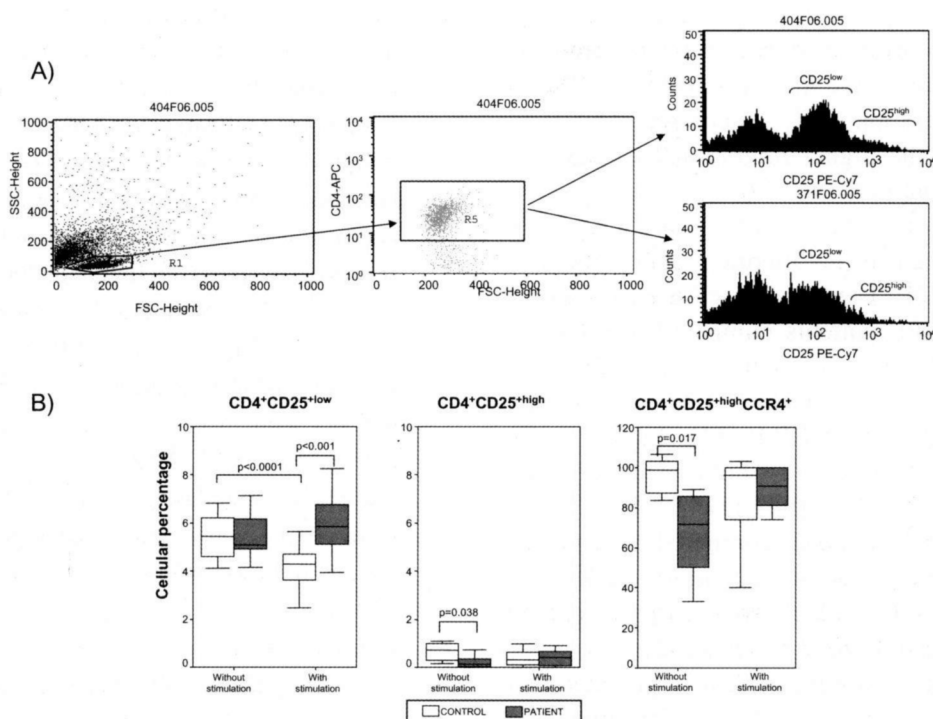


Fig. 5. A) Figure showing gating strategy to analyze the activated CD25^{Low} or regulator CD25^{High} CD4⁺ T lymphocytes. **B)** Box plots represent the comparison of the percentage of activated CD4⁺CD25^{low} T cells or regulatory CD4⁺CD25^{high} and CCR4⁺ expression in the CD4⁺CD25^{high} subpopulation of T cells in patients and controls after 7 days of culture, without and with culprit drug stimulation. Statistical analysis was done using Kruskal-Wallis tests for non-related samples and Wilcoxon test for related samples.

necessary to perform the proliferation methods, which was long enough to differentiate the T_{EM} into specific T_E cells, as has been previously described (24-25, 27-28); and it was probably these latter cells we in fact measured. However, T_{EM} cells showed no differences in patients compared to controls after stimulation with the drug, for which two explanations could exist: the high ability of T_{EM} cells to migrate into target tissue (29) and their capacity to differentiate to T_E cells (24).

The CCR10 chemokine receptor, besides its homing capability, has also been reported to be associated with an effector phenotype, with no expression of CD27 and/or CCR7 (13). We have also reported that during the acute phase of drug-induced MPE there was an increased CCR10 expression in CD4⁺ peripheral blood T cells (6). In the present study, we detected an increase in CCR10 expression in all the subpopulations (total, T_{EM} and T_E) analyzed after drug stimulation, especially in the CD4⁺ T lymphocytes. Moreover, this expression,

in basal non-stimulated cells, was found to be lower in patients compared to controls in all the subpopulations, probably still reflecting the earlier migration of these cells to the skin, as has been described (13).

The evaluation of the migratory capacity of different T-cell subpopulations can be a useful tool to mimic the cutaneous trafficking in NIR to drugs. Here we studied the influence of the two chemokines, CCL17 and CCL27, both of which have been shown to be involved in drug-induced cutaneous reactions (11, 30). We detected a higher migration induced by CCL27 in controls without the drug and in patients with the drug. This is in concordance with the findings for the CCL27 receptor, CCR10, where the expression was higher in controls without drug stimulation, whereas in patients the expression was higher with drug stimulation. No changes were observed with CCL17.

Interestingly, analysis of the migratory capacity of the T_E and the T_{EM} in patients showed that

stimulation with the culprit drug in the presence of CCL27 had two different effects depending on the T-cell subpopulation: the migration of T_E decreased whereas the migration of T_{EM} increased. This is in agreement with the higher migrational capacity of T_{EM} cells to the target tissue (29).

Finally, as we have reported previously (4), the drugs involved in the allergic reaction induced activation in $CD4^+$ T cells, as measured by CD25 expression in MPE patients compared to controls. Regulatory cells can be defined with different phenotypes, some are $CD4^+CD25^{high}$ (21) and some are $CD4^+CD25^{high}CCR4^+$ (31). In this study a significantly lower level in both regulatory cell phenotypes, $CD4^+CD25^{high}$ and $CD4^+CD25^{high}CCR4^+$, was found in MPE patients compared to controls. These low percentages in regulatory cells may contribute to the tolerogenic response to drugs being avoided or reduced in these patients (32).

In conclusion, in this study we demonstrated that the cells involved in maculopapular exanthematic reactions to drugs are effector memory $CD4^+$ T cells, which respond specifically to the culprit drug, where CCR10 is a chemokine receptor strongly involved in skin homing.

ACKNOWLEDGEMENTS

We thank Ian Johnstone for help with the English language version of the manuscript. This work was supported by the FIS Network RIRAAF (RD07/0064) and the Junta de Andalucía (PI-0201/2007).

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