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Effects of carbamazepine and metabolites on IL-2, IL-5, IL-6, IL-10 and IFN-γ secretion in epileptic patients: the influence of co-medication

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Abstract:
Carbamazepine is a widely used anticonvulsive agent. Its metabolic pathway leads not only to the major active metabolite, carbamazepine-10,11-epoxide, but also to minor terminal metabolites such as iminostilbene and acridine. Carbamazepine is usually well-tolerated, but it may lead to rare, but serious, hypersensitive reactions associated with hypereosinophilia. The mechanisms of hypersensitivity reactions to carbamazepine are still largely unknown, and the implications of the cell-mediated immune response (Th1 pathway) or the humoral immune response (Th2 pathway) are still not understood in these reactions. It is also unclear whether the parent drug or its subsequent metabolites are the primary trigger agent. In our study, we performed *ex vivo* experiments to evaluate the stimulation of cytokine secretion by carbamazepine, carbamazepine-10,11-epoxide, iminostilbene and acridine. IL-5, IL-6 and IL-10 were quantified as markers of the Th2 pathway, and IL-2 and IFN-γ were used as markers of the Th1 pathway. Blood samples (n = 24) were obtained from epileptic patients routinely treated with carbamazepine alone or co-treated with lamotrigine or valproate. The concentrations of cytokines in the plasma were determined before and after 3 h stimulation with drugs. We found a significantly positive effect of co-treatment with valproate on the basal level of IL-5 ($p < 0.01$) and IL-10 ($p < 0.05$). IL-5 production increased only in blood stimulated with a high level of acridine (33 μM), whereas IL-6 production was less specifically stimulated ($p < 0.05$). Because IL-5 is the most potent stimulating factor of the eosinophils, we suggest that the potential helper effect of valproate and acridine can lead to hypersensitive reactions to carbamazepine in the context of the humoral immune response.

Key words:
carbamazepine, IL-5, acridine, valproate, lamotrigine, cytokines, epileptic patients

Abbreviations: 2-OH-CBZ – 2-hydroxy-carbamazepine, 3-OH-CBZ – 3-hydroxy-carbamazepine, AI – acridine, AV – valproate, CBZ – carbamazepine, CBZ-EP – carbamazepine-10,11-epoxide, DIHS – drug-induced hypersensitivity syndrome, DiOH-CBZ – 10,11-dihydro-10,11-trans-dihydroxy-carbamazepine, DRESS – drug rash with eosinophilia and systemic symptoms, IM – iminostilbene, LAMO – lamotrigine

Introduction

Carbamazepine (CBZ) is a frequently prescribed first-line drug for the treatment of partial and generalized tonic-clonic epileptic seizures. Among the adverse reactions associated with CBZ, 5% can be classified as idio-

syncratic or hypersensitivity reactions that are classified into two categories: DRESS, for Drug Rash with Eosinophilia and Systemic Symptoms [11, 29], and DIHS, for Drug-Induced Hypersensitivity Syndrome, which represents only a specific subcomponent of DRESS reactions [15]. Such reactions are unpredictable and are associated with variable clinical pictures and high mortality rates related to multi-organ dysfunctions. High eosinophilia ($> 1.5 \times 10^9$ eosinophils/l) is a common hematological disorder [5]. Contemporaneous viral infections and/or reactivations have been known to occur, but it is still unclear whether they are the trigger agent or a consequence of a humoral immunodeficiency (hypogammaglobulinemia) related to DRESS [2]. Nevertheless, the Japanese groups use HHV-6 reactivation as a criterion for the diagnosis of DIHS [15]. Several other drugs are associated with these hypersensitivity syndromes, and most of them are anticonvulsive agents. Although the culprit drugs are not structurally similar, cross-sensitivities were described, albeit without any explanation of the pathogenesis of the reactions [13, 21]. The hypersensitive reactions are an inappropriate and excessive response to exogenous agents that involve the cell-mediated and/or humoral immune response. In any case, the initiation step of the response requires drug identification by the immune system. For decades, drugs were considered too small to be recognized as antigens by themselves if not covalently bound to a protein. This concept is the hapten theory, which is also called the prohapten theory if the covalent binding concerns a metabolite. In contrast, the more recent p-i theory (pharmacological interaction with immune receptors) describes how a small drug could induce an immune response by direct interaction with major histocompatibility complex molecules and/or T-cell receptors rather than by covalently binding a protein [30]. Nevertheless, the two paradigms require a molecular trigger, (i.e., a parent drug and/or its metabolite(s)). Despite many studies, the culprit molecule(s) is (are) not clearly identified in DRESS related to CBZ. Indeed, CBZ is extensively metabolized, and at least 30 different metabolites have been identified [17]. Three principal metabolic pathways for CBZ have been described, as summarized by Breton et al. [7]. The main pathway results in the formation of carbamazepine-10,11-epoxide (CBZ- EP), a pharmacologically active compound with anticonvulsant properties that is subsequently transformed into the inactive 10,11-dihydro-10,11-trans-dihydroxy-carbamazepine (carbamazepine trans-diol, DiOH-CBZ). Wu et al. [33] showed that CBZ, CBZ-EP and DiOH-CBZ could directly activate T-cells; this finding corroborated the p-i theory. The second metabolic route produces hydroxylated compounds (2-OH-CBZ, 3-OH-CBZ) that are further hydrolyzed into the corresponding iminostilbene and subsequently into an iminoquinone by oxidation. Pearce et al. [26–28] argued in favor of the prohapten theory by identifying a reactive iminoquinone intermediate whose metabolic pathway could involve 2-OH-CBZ, 3-OH-CBZ and 2,3-dihydroxy-carbamazepine. The third minor pathway of metabolism leads to the formation of iminostilbene (IM). Although the liver is the major site of drug metabolism, the biological half-life of most reactive metabolites is not sufficient to cause toxicity at distant sites. Not only are leukocytes the major cells involved in the induction of an immune response, but they also exhibit strong oxidizing properties. In the leukocytes, CBZ is converted by myeloperoxidase into a series of metabolites, which are primarily an intermediate aldehyde, 9-acridine carboxaldehyde, acridine (AI) and acridone [10].

In DRESS, it is not clear whether the immune response is humoral or cytotoxic. Because cytokines modulate the orientation of the immune response, Marmurowska-Michałowska et al. [22] investigated the *in vitro* profile of cytokine production on the basis that IL-2, TNF-α, IFN-γ and IL-12 lead to the cell-mediated response (Th1 pathway), whereas IL-4, IL-5, IL-6, IL-10 favor the humoral response (Th2 pathway). Since this report, Lochmatter et al. [19] showed that the measurement of IL-2, IL-5, IL-13 and IFN-γ is the most useful *in vitro* tool for the detection of T-cell sensitization in delayed-type drug hypersensitivity.

In this study, we present the basal production of IL-2, IL-5, IL-6, IL-10 and IFN-γ in the blood of routinely treated epileptic patients and the impact of CBZ, CBZ-EP, AI and IM on the production of these cytokines after *ex vivo* stimulation. Because the patients were treated with CBZ alone or with co-therapies including valproate (AV) or lamotrigine (LAMO), we investigated the potential influence of the regime of treatment on cytokine production.

Materials and Methods

Materials

CBZ, CBZ-EP, AI and IM were purchased from Sigma (Saint Quentin Fallavier, France). HPLC-grade aceto-

nitrile, which was used to dissolve the chemical compounds, was obtained from Merck (Nogent/Marne, France). The concentration of the stock solutions was 10 mM for the four drugs.

Patients

Patients hospitalized in our university hospital (Montpellier, France) were recruited according to a protocol approved by the local Research Ethics Committee. The inclusion criteria were as follows: diagnosis of epilepsy at least one year prior to the study, age between 18 and 90, written informed consent prior to any study-related procedure and well-tolerated and effective treatment after at least one month of CBZ therapy. Co-treatments with AV and LAMO were allowed. The exclusion criteria were as follows: pregnancy and use of non-anticonvulsive medications known to be potential inhibitors/inducers of enzymatic systems.

Blood sampling

Blood samples (15 ml) were collected in heparinized tubes immediately before drug intake (12–14 h after the previous dose). *Ex vivo* experiments were immediately performed.

Ex vivo experiments

The collected blood was divided into aliquots of 500 µl to test the influence of the selected drugs on the secretion of IL-2, IL-5, IL-6, IL-10 and IFN-γ. Appropriate mixtures of stock solutions and saline solution were added to the blood to obtain the following final concentrations: 33 µM and 100 µM for CBZ; 10 µM and 33 µM for CBZ-EP, AI and IM. The final volume added to the blood for each experiment was 5 µl (i.e., 1% of biological volume). Blood aliquots were then incubated for 3 h with agitation in unstoppered 5 ml glass tubes at 37°C and pH values, measured each hour, remained at physiological levels throughout the experiments (pH, 7.4 ± 0.1). IL-2, IL-5, IL-6, IL-10 and IFN-γ levels were determined just before the *ex vivo* experiments (basal level) and at the end of the incubation. Because the drugs were diluted in acetonitrile, the effect of the solvent on the stimulation of the cytokines was evaluated by comparing the cytokine activation with that obtained under the same conditions with blood spiked with a sterile saline solution for intravenous use. The incubation time was extended to 6 h for these experiments. The plasmatic concentration of CBZ was measured before the incubations for each patient.

Analytical method

The CBZ concentrations were initially assessed in plasma with the carbamazepine PETINIA method for Xpand automate from Siemens (New York, USA).

The concentrations of IL-2, IL-5, IL-6, IL-10 and IFN-γ were determined in whole blood without PBMC fractionation or culturing with the commercialized FastImmune Cytokine System from Becton Dickinson (New Jersey, USA).

Statistical analysis

The basal level of cytokine production was estimated from the median and range of values observed at the beginning of the incubation. The effect of acetonitrile on the stimulation of cytokines was evaluated by comparing the level of cytokine production with that obtained under the same conditions with blood spiked with a sterile saline solution using a non-parametric paired Wilcoxon test.

To remove any solvent effect, all determinated cytokine concentrations were corrected by subtracting the concentrations obtained under the same conditions with acetonitrile. To compare the impact of CBZ, CBZ-EP, AI and IM on cytokine production after *ex vivo* stimulation, a non-parametric Wilcoxon test for paired samples was performed. The cytokine production was also compared between patients treated with CBZ alone or those treated with co-therapy (AV or LAMO) using a Mann-Whitney test. We considered $p < 0.05$ to be significant in all statistical analyses.

Results

Patients

Twenty-four patients were recruited. The sex ratio of the population was 8 females and 16 males. The median ages were 43 years (range, 30–55 years) and 36 years (range, 21–66 years) for females and males, respectively. Eighteen patients were treated with CBZ

alone; six were treated with a combination of CBZ and AV; and six others were treated with CBZ and LAMO. The CBZ levels were between 1.8 and 17.1 mg/l (median, 8.5 mg/l). More specifically, most of the patients were in the therapeutic range for CBZ, except for two patients who were below and three who were above.

Basal profile of the cytokine production

The comparative profile of basal cytokine production for the different therapies is presented in Figure 1. We found very few values regarding normal values for cytokines. In accordance with Aihara et al. [1], we chose the value 0.1 pg/ml as a positive threshold to measure any increase in cytokine production. Thus, basal production levels appeared to increase for all cytokines in the CBZ-treated patients; these production levels displayed a smaller increase in patients co-treated with LAMO. IL-2 was the most abundant cytokine found in the blood, regardless of the type of treatment, and its concentrations always exceeded the positive threshold (Fig. 1A). Moreover, the minimum level of IL-2 was higher than the maximum values of all other cytokines in patients with bi-therapy (Fig. 1A–E). Conversely, the distribution of the basal levels of IL-6 and IFN-γ overlapped a part of those of IL-2 in the patients treated only with CBZ (Fig. 1A, C and E), but IL-2 was the most abundant cytokine in each individual profile (data not shown). IL-5 and IL-10 levels were low for all treatment regimens: the median values ranged between 0.03 and 0.97 pg/ml. As shown in Figure 1B, we observed a highly significant augmentation of the basal production of IL-5 ($p = 0.003$) in the patients treated with the therapeutic combination of CBZ and AV. A less significant effect was seen for IL-10 basal production ($p = 0.02$) (Fig. 1D). The group of patients treated only with CBZ had a larger dispersion of IL-6 and IFN-γ concentrations than the groups of patients with bi-therapy, but this difference was not statistically significant (Fig. 1C and 1E).

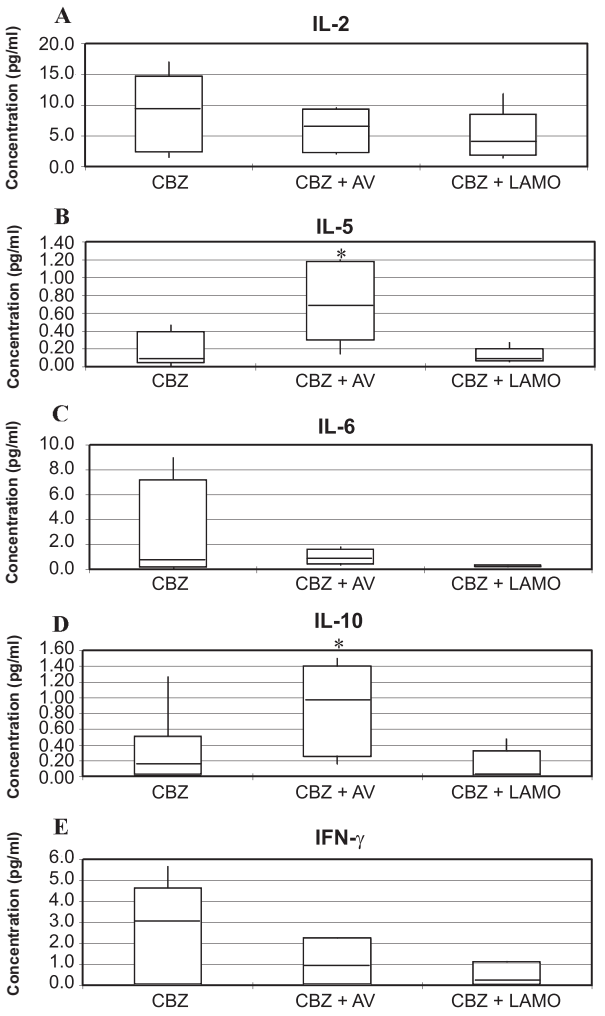

Fig. 1. Basal profiles of IL-2 (**A**), IL-5 (**B**), IL-6 (**C**), IL-10 (**D**) and IFN-γ (**E**) in the plasma of treated epileptic patients. For each cytokine, the box plot diagrams (min, median, interquantiles 10 and 90, max) compare the basal profiles of cytokine production in 3 groups: patients treated with CBZ alone (CBZ), patients treated with both CBZ and AV (CBZ + AV) and patients treated with both CBZ and LAMO (CBZ + LAMO). All of the concentrations are expressed in pg/ml. Co-medication with AV appeared to increase the basal production of IL-5 and IL-10 in a statistically significant manner, * $p < 0.05$

Cytokine production after *ex vivo* stimulation

The profiles of cytokine production were not statistically different between acetonitrile and saline in the experiments used to evaluate the effect of the solvent (Fig. 2). The concentration distributions of IL-2, IL-5, IL-10 and IFN-γ shared the same pattern: more than 10% of the levels were below the detection limit of the method, and the distributions of the values were constrained regardless of the conditions for each cytokine. Most of the concentrations were below 2.0, 3.0, 20 and 30 pg/ml for IL-5, IL-10, IFN-γ and IL-2, respectively. Conversely, the pattern of IL-6 production varied substantially. The concentrations were low at the beginning of the incubation, but very high levels were measured at the end of the incubation. Nevertheless, such values were reached by less than 10% of each experimental condition; and this response was

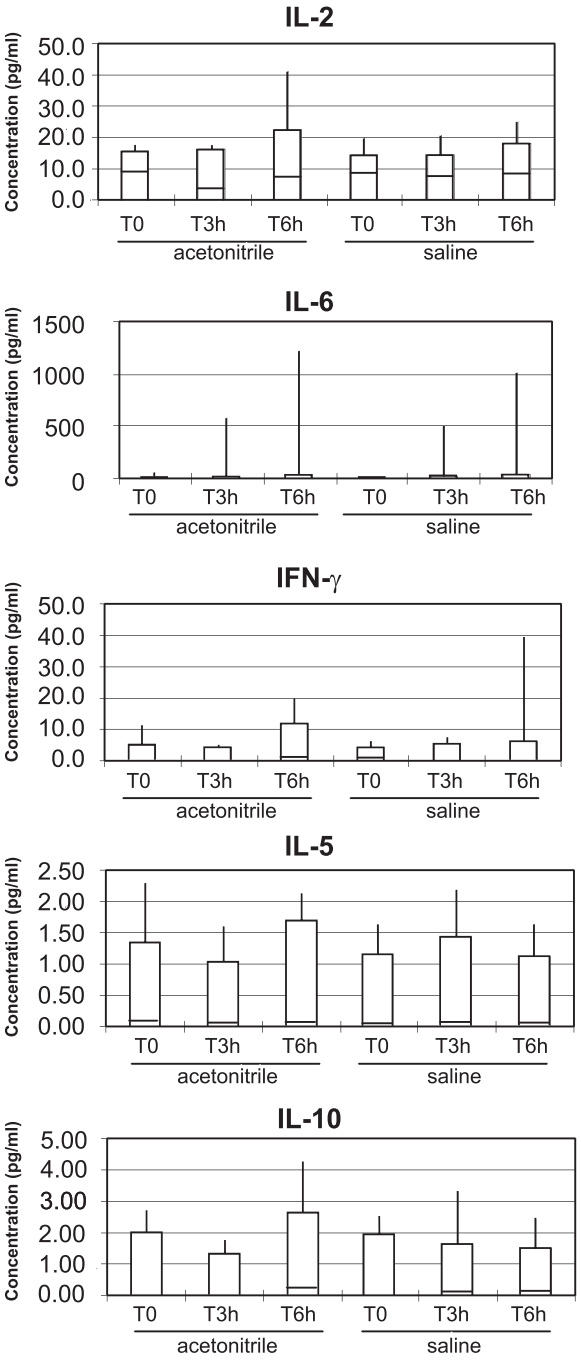

Fig. 2. Effect of the solvent on cytokine production. The effect of the solvent was assessed at the beginning of the stimulation (T0), after 3 h (T3h) and after 6 h (T6h) for acetonitrile and saline. The box plots present the min, median and max values; the inner boxes cover 80% of all values. All concentrations are expressed in pg/ml

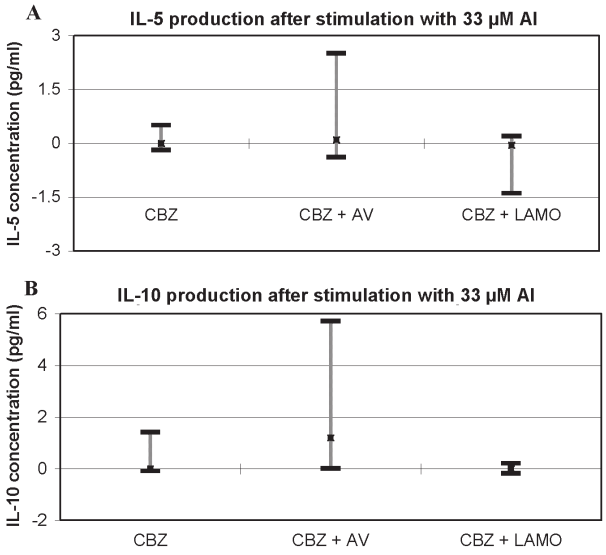

Fig. 3. Net production (deduced from the basal level) of IL-5 (**A**) and IL-10 (**B**) at the end of the ex vivo 3 h incubation of blood from epileptic patients stimulated with 33 µM AI. Median (■), min and max values are reported

comparable to those observed for the saline and acetonitrile experiments.

Table 1 summarizes the results of the statistical analysis. A strong variation in IL-6 levels was observed under all conditions, except when stimulation was performed with a low level of IM. The range of the median concentrations was between 0.94 pg/ml with 10 µM AI and 4.29 pg/ml with 33 µM IM, which corresponded to a relative increase from 16 to 91% compared to the solvent effect alone. Interestingly, stimulation with a low level of CBZ-EP resulted in an increase in IL-6 production compared to the solvent effect, whereas a decrease (–91%) was observed with a high level of CBZ-EP. IL-5 production was only observed when the higher level of AI was administered. The median value of the measured net concentrations (deduced from the solvent effect) was 1.06 pg/ml, which corresponded to a 33% increase compared with the solvent value under the same experimental conditions. No variations in the production of IL-2, IL-10 and IFN-γ were observed. A subgroup analysis of the variation in IL-5 and IL-10 production before and after stimulation with 33 µM AI did not show any significant difference between the blood samples of patients co-treated with both AV and CBZ compared to the others (Fig. 3).

Discussion

In this study, we have chosen to compare each patient with him- or herself in the restricted context of pa-

Tab. 1. Results of the statistical analysis of the *ex vivo* experiments. For each tested drug, the non-parametric Wilcoxon test for paired samples was applied to determine whether there was a significant difference in cytokine production compared to the corresponding solvent value after 3 h of incubation. The significant results are indicated with asterisks, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Direction of change is indicated: ↑ – for increase or ↓ – for decrease. n.s. – not significant

Tested drug	CBZ		CBZ-EP		AI		IM	
Stimulation level	33 μM	100 μM	10 μM	33 μM	10 μM	33 μM	10 μM	33 μM
IL-6	*** ↑	*** ↑	** ↑	*** ↓	** ↑	** ↑	n.s.	*** ↑
IL-5	n.s.					* ↑	n.s.	
IL-2, IL-10, IFN-γ	n.s.							

tients chronically exposed to CBZ. We did not include a control group in our study because of the inherent scientific difficulties that would have impeded the design of an efficient comparator. Indeed, the best control group for our experiments would have been constituted of fresh blood samples from recently diagnosed and still-untreated epileptic patients for whom treatment with carbamazepine was required, as opposed to healthy volunteers, to take into account the change in basal cytokine secretion (related to a stimulation process) in epileptic patients [23, 35]. Regarding the need to perform our *ex vivo* experiments on fresh specimens with the highest reproducibility, the time of the inclusion step for this control group would have led to a longer study with many isolated stimulation assays for control samples. The alternative, a control group composed of healthy volunteers, was also unattractive because the length of the exposure to carbamazepine influences the response of the immune cells [22, 35], and the experiments would have required the healthy volunteers to be treated for one month. However, ethical considerations did not allow the exposure of healthy volunteers to CBZ in this context. Finally, the comparison of results obtained in patients chronically exposed to carbamazepine with those obtained from healthy unexposed volunteers would have not permitted us to distinguish between the effects related to CBZ or those related to epilepsy.

Młodzikowska-Albrecht et al. [23] previously showed that IL-2 secretion increases during CBZ therapy (Fig. 1A). Verrotti et al. [32] reported an increase in IL-2 in the PBMC of healthy volunteers treated with CBZ, but not in those of patients treated with AV when compared to the basal value before drug intake. Pacifici et al. [25] observed a similar result in the PBMC of treated epileptic patients.

The increase in IL-6 secretion during CBZ therapy (Fig. 1C) has already been described [23]. Shiah et al. [31] observed that AV therapy induced an increase in the plasma levels of IL-6 after 7 days of valproate treatment in ten healthy volunteers. The authors found IL-6 production to be significantly higher in patients treated with CBZ and AV than in controls; the levels of production were comparable for both treatments. Ichiyama et al. [14] demonstrated that AV inhibits IL-6 production, but their *in vitro* studies were restricted to the inhibition of the NF-κB pathway in human monocytic leukemia cells. As far as we know, no data regarding cytokine production are available for LAMO. The results obtained after incubation with CBZ and its metabolites demonstrated the marked ability of these molecules to impact IL-6 levels when compared to incubation in acetonitrile under the same conditions (Tab. 1). Strangely, the upper level of CBZ-EP (within the therapeutical range) appeared to reduce the IL-6 concentrations relative to the solvent, but an increase was observed in almost all other experiments. Thus, the anti-epileptic effect of this main active metabolite of CBZ could be explained, at least partially, by an anti-inflammatory effect linked to the inhibition of IL-6 production. One can attribute non-specific IL-6 stimulation to experimental manipulations, but this perspective does not explain the inhibitory effect of high levels of CBZ-EP. An augmentation of IL-6 production in patients treated with CBZ should result both from an intrinsic effect of CBZ itself and its metabolites, even if CBZ-EP has an inhibitory effect. Because CBZ-EP depends on the activity of the cytochrome P450 3A4 isoenzyme, modulation of IL-6 production could occur in polymedicated patients or in patients with strong CYP3A4 activity. Furthermore, Gidal et al. [12] suggested that IL-6 itself

could affect the activity of this isoenzyme, thus entailing a more complex inter-regulation.

Marmurowska-Michałowska et al. [22] incubated the PBMC of 16 healthy volunteers for 72 h with a solution of CBZ in DMSO (final concentration, 1 μM). Two series of controlled-experiments were performed: experiments with unstimulated PBMC and experiments with PMBC stimulated with a mixture of hemagglutinin and LPS. IL-2, IL-10 and IFN-γ were quantified in these experiments and in ours. In our experiments, we chose to use acetonitrile rather than DMSO because we observed a stronger solvent effect for DMSO on IL-10 production after an incubation performed for 3 h (data not shown). We did not observe any effect of CBZ or its metabolites on IL-2, IL-10 and IFN-γ production in our experiments whose samples were not stimulated with PHA + LPS (Tab. 1). Marmurowska-Michałowska et al. [22] observed an increase in IL-10 production after incubation with CBZ in both stimulated and unstimulated PBMC. No significant difference in IL-10 levels was observed in our experiments, including the subgroup of CBZ-AV treated patients ($p = 0.08$). The absence of significance may be due to insufficient statistical power in our study. However, because the number of samples is of the same order of magnitude in the statistical analysis, we believe that this discrepancy arose from the helper effect of DMSO on IL-10 production in Marmurowska-Michałowska's study. We only found indirect bibliographic data regarding the influence of AV on IL-10 production in a subgroup of patients by Boufidou et al. [6]. Further studies are required to confirm a specific effect of CBZ-AV co-therapy on IL-10 production.

IL-5 is the most potent colony-stimulating factor of the eosinophils, favoring their maturation and activation, but as far as we know, experimental modulation of its production by CBZ treatment has not been investigated. In our study, basal levels of IL-5 were significantly more elevated in the blood of patients treated with both CBZ and AV compared to those of patients treated with CBZ alone or with CBZ-LAMO. Makis et al. [20] observed a double clinical impact of AV on the production of IL-5: AV increased the level of IL-5 and led dose-dependently (but not equally in all patients) to an optimal therapeutic response and/or to eosinophilia. Several clinical observations of cross-sensitivity between CBZ and AV leading to hypersensitivity syndrome were published [3, 8, 9, 34]. Interestingly, Yun et al. [34] described the clinical case of a Korean epileptic woman safely treated with AV who then suffered from skin lesions when treated with CBZ alone and finally developed a DRESS when AV was reintroduced alone. The eosinophilia was intense in all of the episodes of the adverse reaction, and a circulating auto-antibody was identified. These observations suggest that IL-5 is involved in the activation of the Th2 immunological pathway. However, cross-sensitivity is not always observed. Aouam et al. [2] described the case of a well-tolerated treatment with AV taken before and after a DRESS syndrome to CBZ (which was associated with a HHV6 primary infection). The occurrence of a DRESS syndrome was also observed in a complex clinical context with AV, ethosuximide and the reactivation of HHV6 infection [9]. Unfortunately, no determinations of the metabolites of CBZ were performed in these studies. We showed that CBZ could activate the production of IL-5 by AI (Tab. 1). Whether such a level could actually be attained in blood is questionable, even if high local concentrations may be present after accumulation in the white cells producing IL-5. Moreover, AI is not found in all CBZ-treated patients [7], but hypersensitive adverse reactions are also rare. An increase in IL-5 production after stimulation with high AI concentrations implies the presence of a threshold level. Interestingly, activation of T-cells also depends on a threshold level that is lower if the T-cell has been previously sensitized. In reactive patients, such a sensitization could be due to viral infection and the cross-reactivity of the viral antigen with CBZ (in the frame of the p-i concept without requiring a hapten). The high basal IL-2 level caused by the anticonvulsive immunologic activity of CBZ could maintain a permanent alert signal (Fig. 1A). AI could become a trigger metabolite for IL-5 production in favorable circumstances that diminish the secretion threshold of the T-cells (decrease in immunity after asymptomatic viral reactivation, deficiency in regulatory T-cells or increase in cytokine production due to an anticonvulsive drug and/or banal aggression). A lower threshold could also be due to AV because, as we demonstrated in this study, co-treatment with AV increases the basal production of IL-5 and IL-10 (Fig. 1) and tends to facilitate this stimulation by AI (Fig. 3). Because Bernus et al. [4] showed that AV diminishes CBZ-EP catabolism without increasing CBZ levels, we postulate that AV is able to divert the CBZ major metabolism pathway to minor pathways, such as those for AI formation, and then facilitate IL-5 production. From our re-

sults, increases in CBZ-EP due to AV co-treatment could also limit IL-6 production and counteract inflammation processes and adverse reactions. This subtle equilibrium may explain the different patterns of cross-reactivity in the clinical reports quoted above. LAMO was identified as an important etiologic agent of the anticonvulsive hypersensitivity syndrome, but co-treatment with AV is frequent [21]. AV was also suspected to increase the risk of allergic skin rash with lamotrigine [16, 18]. The inhibitory effect of AV on the metabolism of LAMO [24] is probably involved, which is an argument to consider LAMO itself as the trigger compound rather than its main metabolites.

An imbalance in the production of cytokines is undoubtedly one of the triggers of hypersensitive adverse reactions to anti-convulsive agents cumulatively through their own impacts on cytokine production, the basal disturbance due to epilepsy and accidental events such as viral infections/reactivations. The complexity of the mechanism may be further amplified by the effect of drug metabolites on cytokine production, including polymorphism in metabolic capacity and potential feedback loops of the cytokines on this metabolism. Our data show potential strong interactions between CBZ metabolism, AV co-treatment, IL-5 production and DRESS. CBZ-AV therapeutical association could increase the risk of DRESS, which has been illustrated by several clinical case-reports. Nevertheless, more data from patients suffering from acute DRESS are required. To conclude, considering these data from the perspective of the imbalance between the Th1 and Th2 immunological pathways and relative cytokines, CBZ and its metabolites seem to favor the humoral immune response, which is supported by the findings that IL-5 and IL-10 favor Th2 polarization and IL-6 stimulates plasmocyte differentiation. According to Pearce's group [26], Th2 activation could lead to auto-antibody production [34].

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