

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Multiple drug hypersensitivity: normal Treg cell function but enhanced *in vivo* activation of drug-specific T cells

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Keywords

CD25^{dim}; drug allergy; drug rash with eosinophilia and systemic symptoms; T cells; T regulatory cells.

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Abstract

Background: Up to 10% of patients with severe immune-mediated drug hypersensitivity reactions have tendencies to develop multiple drug hypersensitivities (MDH). The reason why certain individuals develop MDH and the underlying pathomechanism are unclear. We investigated different T cell subpopulations in MDH patients and compared them with patients allergic to a single drug and with healthy controls (HC).

Methods: We analyzed the *in vitro* reactivity of peripheral blood mononuclear cells from MDH patients ($n = 7$), patients with hypersensitivity to a single drug (monoallergic, $n = 6$), and healthy controls (HD) ($n = 6$) to various drugs (mainly antibiotics and antiepileptics). By depleting and selectively re-adding CD4⁺ CD25^{bright} T cells (T regulatory cells, Treg), their effect on drug-specific T cell reactivity was analyzed. The phenotype of reacting T cells was determined *ex vivo* by staining for markers of activation (CD38) and cell exhaustion (PD-1).

Results: No functional deficiency of Treg cells was observed in all drug-allergic patients. Drug-reactive T cells from MDH patients were found in the CD4⁺ CD25^{dim} T cell fraction and showed enhanced CD38 and PD-1 expression, while those from monoallergic patients reside in the resting CD4⁺ CD25^{neg} T cell fraction.

Conclusion: In patients with MDH, the drug-reactive T cells are contained in an *in vivo* pre-activated T cell fraction. Therefore, they may show a lower threshold for activation by drugs. The reason for this *in vivo* T cell pre-activation needs further investigations.

Drug allergies are a common and complex problem in clinical practice (1–3). A subgroup of patients with drug allergies react to two or more chemically distinct drugs. This was first recognized by Sullivan (4) in 1986 who described patients with multiple reactions to chemically distinct antibiotics and coined the term ‘multiple drug hypersensitivity’ (MDH). However, it remained a purely clinical description based on history and observation alone, while the pathomechanism of this syndrome remained unclear. This caused some confusion concerning terminology, and in subsequent years, different clinical reactions have been described as MDH syndrome, including multiple drug intolerance to structurally distinct

nonsteroidal anti-inflammatory drugs (5, 6), which is not immune mediated but related to their pharmacological properties (cyclooxygenase inhibition).

Our group has identified a number of patients who developed reactions to two or more chemically distinct drugs (7, 8). The majority of these patients presented with severe forms of drug allergy including DRESS/DIHS (drug rash with eosinophilia and systemic symptoms, also called drug-induced hypersensitivity syndrome). The immunological basis of these MDH was demonstrated by positive skin or *in vitro* tests (8). In this study to investigate immunological mechanisms of MDH, all patients had a positive *in vitro* lymphocyte trans-

formation test (LTT) (9), which is the most widely used assay to detect T cell sensitization to drugs.

It is possible that patients with MDH develop an enhanced responsiveness to drugs because of a deficient tolerance mechanism. Previously, Cavani et al. (10) observed that Treg cells from nickel-exposed nonallergic, but not nickel-allergic, individuals suppress both naïve and memory nickel-specific T cell responses *in vitro*. In systemic drug allergy, Takahashi et al. (11) recently reported a significant expansion of functional Treg cells during the acute phase in DRESS patients, which was followed by a deficient Treg cell function after recovery. Thus, the development of an exaggerated immune response to drugs in MDH could be a consequence of a defective or reduced suppressive activity of Treg cells. However, it is also possible that MDH is associated with an over-activation of drug-specific T cells by incriminated or bystander drugs.

To address these possibilities, we compared the activation of different T cell subpopulations in MDH patients, patients allergic to a single drug (termed monoallergic), and healthy controls (HC) and analyzed the influence of Treg depletion on drug reactivity. We demonstrate for the first time that drug-reactive T cells of MDH patients are localized in a distinct T cell fraction compared with monoallergic patients, while the Treg function was preserved in all drug-allergic patients.

Methods

Donor characteristics

The clinical characteristics of seven patients with diagnosed MDH syndrome are summarized in Table S1A. In five patients, the syndrome started with DRESS/DIHS. Subsequent clinical reactions differed in intensity and organ involvement, but all reactions were T cell mediated. The clinical characteristics of the six monoallergic patients included in the study are summarized in Table S1B. In both groups, the eliciting drug was defined according to typical medical history of drug allergy and positive *in vitro* LTT. Drug-exposed individuals ($n = 6$) without a history of adverse drug reactions (ADRs) served as controls.

Blood samples were taken in full clinical remission. All participants gave informed consent, and the study was approved by the local ethical committee.

Drugs, antigens, and culture conditions

Drug concentrations studied were nontoxic in peripheral blood mononuclear cells (PBMCs) and stimulated lymphocytes in sensitized individuals, but not in HC (data not shown). The concentrations of drugs used for all studies are summarized in Table S2. PBMCs were cultured in RPMI-1640 (Gibco; Invitrogen, Basel, Switzerland) supplemented with 10% pooled heat-inactivated human AB serum (Swiss Red Cross, Bern, Switzerland), 2 mM L-glutamine (Gluta-Max; Gibco), 25 µg/ml holo-transferrin (Sigma-Aldrich, Buchs, Switzerland), 100 µg/ml streptomycin, and 100 IU/ml penicillin (Gibco).

Isolation of PBMCs and T cell subsets from blood samples

Immunomagnetic beads were used to isolate different cell subsets from PBMCs (see Fig S1, all from StemCell Technologies, Grenoble, France). In brief, to prepare antigen presenting cells (APCs), CD3⁺ T cells were depleted from PBMCs using human CD3 selection kit[®]. CD4⁺ T cells were enriched using CD4⁺ T cell enrichment kit[®]. To prepare Treg cell-depleted CD4⁺ T cells, CD25^{bright} cells were depleted from CD4⁺ T cells using anti-CD25 immunomagnetic beads. The T cells depleted of CD25^{bright} cells consisted of CD4⁺ CD25^{neg} and CD4⁺ CD25^{dim} subsets: the CD4⁺ CD25^{dim} were obtained by longer incubation with the anti-CD25 beads. Thus, CD4⁺, CD4⁺ CD25^{bright}, CD4⁺ CD25^{dim}, and CD4⁺ CD25^{neg} subsets were separated and analyzed. All kits were used according to the manufacturer's protocol (Version 2.0.2, 2008, Easy Sep[®]; StemCell Technologies).

Antibodies and flow cytometry analysis

Freshly isolated PBMCs (2.5×10^5) were cultured in duplicate in 96-well U-bottom plates in the presence of indicated drug concentrations or tetanus toxoid (TT). The antibody assays used for flow cytometric analysis including their fluorochromes are summarized in Table S3.

Cell sorting

To obtain FACS (fluorescence activated cell sorting)-sorted T cell subsets, the freshly isolated PBMCs were first predepleted using immunomagnetic beads (CD4⁺ T cell enrichment kit, Easy Sep[®]; StemCell Technologies). The CD4⁺ T cells were subsequently stained for anti-CD4-PerCP and anti-CD25-PE, and the subpopulations CD4⁺ CD25^{neg}, CD4⁺ CD25^{dim}, and CD4⁺ CD25^{bright} were isolated using a FACSaria (BD Biosciences, San Jose, CA, USA).

T cell proliferation assay

The purified T cell subsets mentioned earlier (80 000 cells/well) and autologous γ -irradiated APCs (20 000 cells/well) were cultured in 96-well U-bottom plates in the presence of culprit and uninvolved drugs as well as TT as positive control. CD4⁺ CD25^{bright} T cells (8000 cells/well) were added, if indicated, either to the CD4⁺ CD25^{dim} T cell or to the CD4⁺ CD25^{neg} T cell fractions. After 6 days, 0.4 µCi ³H-thymidine (PerkinElmer, Boston, MA, USA) was added and after overnight incubation, proliferation was measured by scintillation counting.

Generation of specific T cell clones (TCCs)

T cell lines and TCCs were generated from one MDH patient with reactions to carbamazepine, phenytoin, and phenobarbital. Clones were generated by stimulating PBMCs with carbamazepine (25 µg/ml), phenytoin (25 µg/ml), and phenobarbital (25 µg/ml) for 14 days prior to serial dilution and

repetitive mitogen-driven stimulation as described previously (12). To test the specificity of the clones, T cells (0.5×10^5) were incubated with irradiated autologous APCs (0.1×10^5) and titrated concentrations of the three drugs mentioned previously (1–100 $\mu\text{g/ml}$). After 48 h, ^3H -thymidine was added and proliferation was measured 16 h later by scintillation counting.

Statistical analysis

Nonparametric Kruskal–Wallis test was used to measure significance. Values of $P < 0.05$ were considered significant.

Results

Characterization of patients

All drugs to which the MDH patients had a confirmed allergic reaction elicited an increased expression of four different activation markers (CD25, CD69, CD71, and HLA-DR) on CD4^+ and CD8^+ T cells after *in vitro* stimulation. The T cells did not react to uninvolved drugs (Fig. 1, data shown for CD69 and CD71 upregulation on CD4^+ T cells; see also Fig. S1 for CD69 and CD71 upregulation on CD8^+ T cells). The kinetics of activation marker expression was similar in MDH and monoallergic patients (data not shown). To exclude cross-reactivity at the individual T cell receptors, TCCs were generated from one additional MDH patient reacting to carbamazepine, phenobarbital, and phenytoin at different time points. To each drug, separate non-crossreactive TCCs could be generated (Table S4).

The number of circulating CD4^+ CD25^+ Foxp3^+ Treg cells does not differ between MDH and monoallergic patients and healthy controls

A possible explanation for MDH would be a deficiency of Treg cells. We therefore assessed the frequency of Treg cells in peripheral blood according to Foxp3 and CD25 expression, which revealed no difference between MDH patients, monoallergic patients, and control individuals (Fig. S2).

Difference in the influence of CD25^+ T cell depletion between monoallergic and MDH patients

Given the comparable numbers of circulating Treg cells between the groups studied, we examined whether a functional defect in these cells in MDH patients exists by assessing their capacity to suppress drug-stimulated T cells in the same individual.

Total CD4^+ T cells and two CD4^+ T cell subsets were isolated according to their CD25 expression by immunomagnetic sorting (CD4^+ CD25^{neg} and CD4^+ $\text{CD25}^{\text{bright/dim}}$ T cells).

Proliferation assays on the total and the above-mentioned sorted CD4^+ T cell fractions were stimulated using

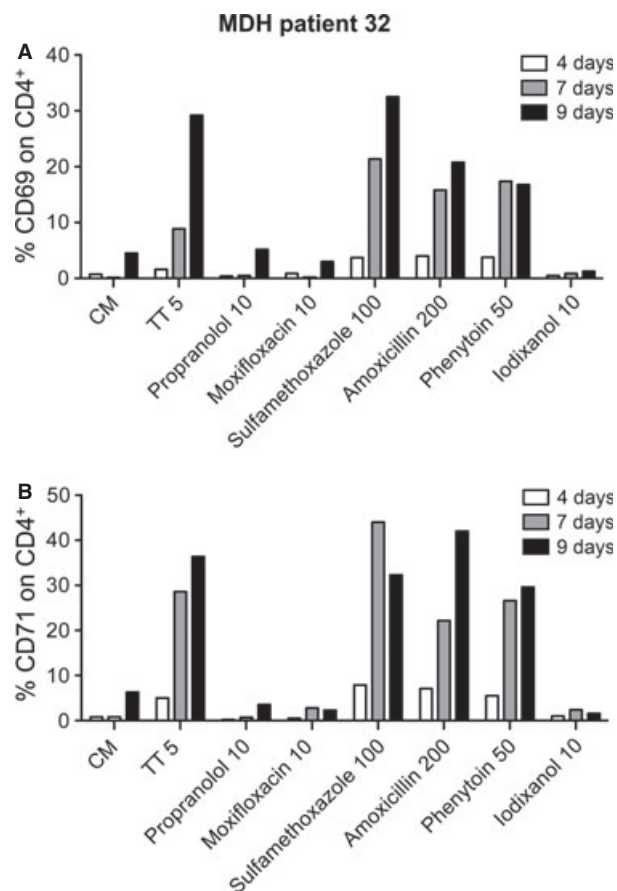


Figure 1 Upregulation of T cell activation markers CD69 and CD71 on CD4^+ T cells of multiple drug hypersensitivities (MDH) patient 32 after drug stimulation. Kinetics of CD69 (A) and CD71 (B) upregulation is shown at three different time points (4, 7, and 9 days). Data are representative for two further MDH patients tested (303 and 556). CM, culture medium only; TT, tetanus toxoid (positive control). Concentrations are represented in $\mu\text{g/ml}$.

a set of drugs (culprit and uninvolved). First, the monoallergic patients showed an enhanced proliferation after depleting CD4^+ $\text{CD25}^{\text{bright/dim}}$ T cells ($=\text{CD4}^+$ CD25^{neg}) compared with the proliferative response of their total CD4^+ T cells. Adding back the autologous CD4^+ $\text{CD25}^{\text{bright}}$ T cells (CD4^+ CD25^{dim} T cells were excluded in a second purification step according to the Easy Sep[®] protocol, Fig. S2) resulted in a proliferative response comparable with that in the total CD4^+ T cells (Fig. 2B). The proliferation to TT stimulation in HC behaved likewise (Fig. 2A).

Second, the cells of MDH patients reacted differently: we consistently observed a reduced proliferative response after depleting CD4^+ $\text{CD25}^{\text{bright/dim}}$ T cell, which could not be compensated by adding back CD4^+ $\text{CD25}^{\text{bright}}$ T cell (Fig. 2 C). This led us to the assumption that the CD4^+ CD25^{dim} T cell subfraction is essential in MDH patients.

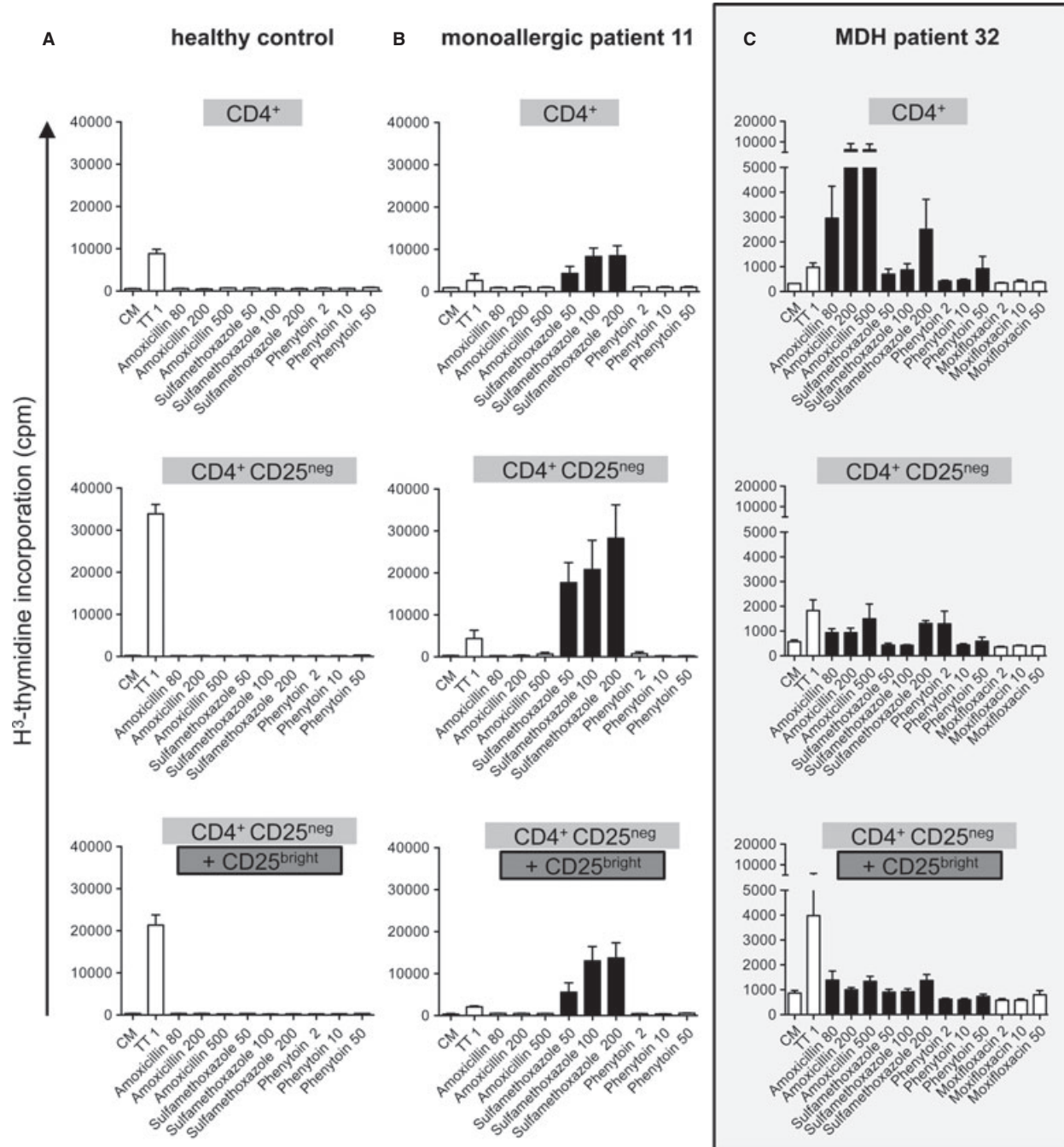


Figure 2 Difference in the influence of CD4⁺ CD25^{bright/dim} T cell depletion on drug-specific T cell reactivity between healthy controls ($n = 6$) and monoallergic patients ($n = 6$) compared with multiple drug hypersensitivities (MDH) patients ($n = 7$). Representative proliferation assays using different CD4⁺ T cell fractions from a healthy control (A) monoallergic patient 11 (B) and MDH patient 32 (C). After immunomagnetic sorting, the different T cell fractions were stimulated with different drugs in quintuplicates and H³-thymidine incorporation is shown as mean with SEM. CD25^{dim/bright} cell depletion

resulted in an enhanced drug-specific T cell reactivity, which returned to the initial level after adding back the CD25^{bright} T cell fraction in monoallergic patient. In contrary, the MDH patient showed diminished drug-specific T cell reactivity without influence of adding back the CD25^{bright} T cells. Culprit drugs are indicated as black bars, and uninvolved drugs and controls as white bars. CM: culture medium only; TT, tetanus toxoid (positive control). Concentrations are represented in $\mu\text{g/ml}$.

In MDH patients, the drug-reactive T cells are found within the CD4⁺ CD25^{dim} population

We further investigated in detail the CD4⁺ CD25^{dim} T cell subfractions, which were separated according to the Easy Sep[®] protocol (Fig. S3). In contrast to the monoallergic

patients, the drug-reactive T cells in MDH patients were consistently found within the CD4⁺ CD25^{dim} and not in the CD4⁺ CD25^{neg} T cell fraction (Fig. 3 depicts results of patient 32 in detail, and Fig. 4 summarizes the results of the remaining six MDH patients). The addition of the CD4⁺ CD25^{bright} T cell fraction to these CD4⁺ CD25^{dim} T

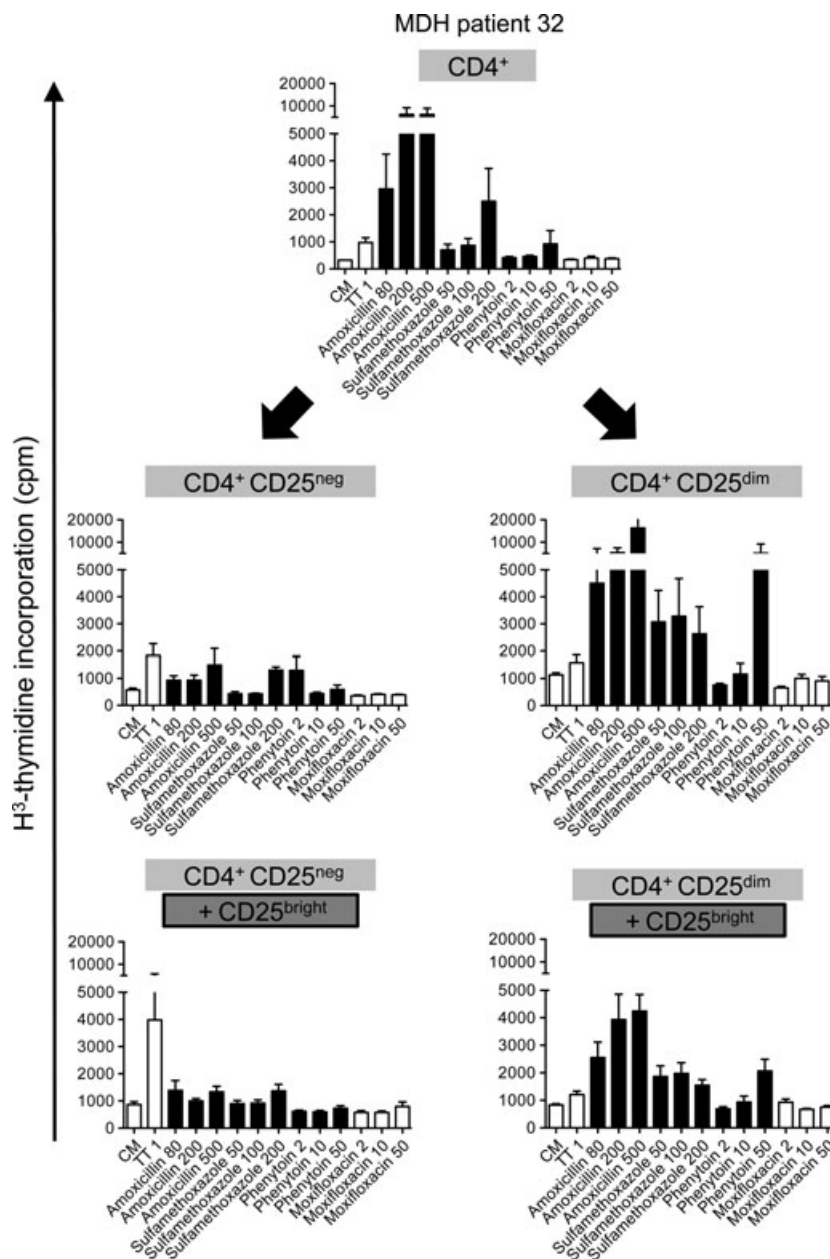


Figure 3 In multiple drug hypersensitivities (MDH) patients, the drug-specific T cells are found within the CD4⁺ CD25^{dim} T cell fraction and are suppressed by the addition of CD25^{bright} T cells. Representative proliferation assays using different CD4⁺ T cell fractions from MDH patient 32. In addition to the T cell fractions used in Fig. 2, CD4⁺ CD25^{dim} T cells were isolated by immunomagnetic cell

sorting and again stimulated with different drugs. Interestingly, the drug-reactive T cells were found within this so far undefined T cell fraction. Culprit drugs are indicated as black bars, and uninvolved drugs and controls as white bars. CM, culture medium only; TT, tetanus toxoid (positive control). Concentrations are represented in µg/ml.

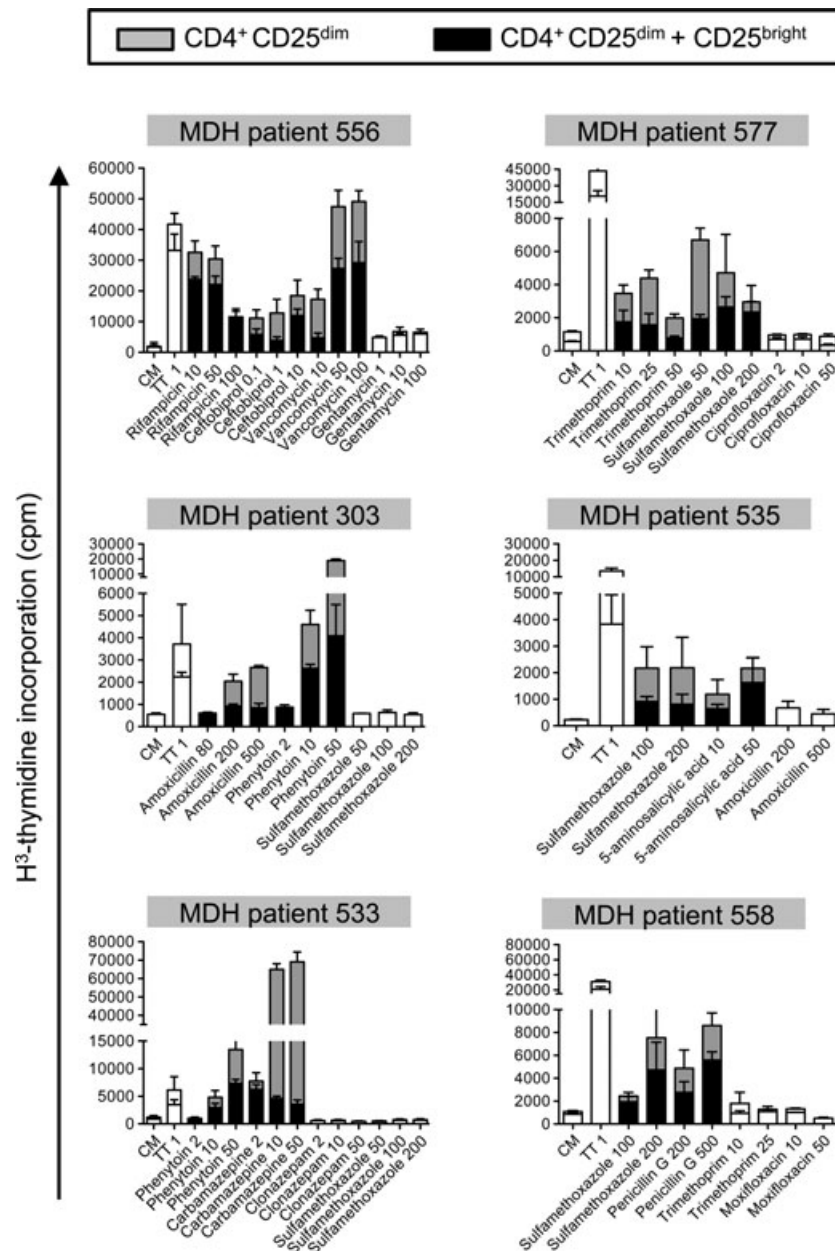


Figure 4 Summarized data of the remaining multiple drug hypersensitivities (MDH) patients ($n = 6$) in analogy to Fig. 3. All MDH patients behaved according to the patient shown in Fig. 3. Drug-reactive CD4⁺ CD25^{dim} T cells are indicated as gray bars, and the

results after addition of the CD25^{bright} T cell fraction as black bars. CM, culture medium only; TT, tetanus toxoid (positive control). Concentrations are represented in $\mu\text{g/ml}$.

cells then resulted in a decreased response to the culprit drugs comparable with the effect of CD4⁺ CD25^{bright} T cell repletion on the CD4⁺ CD25^{neg} T cell fraction in monoallergic patients.

FACS-sorted T cell fractions show the same response pattern

To verify these findings of differently reacting T cell subfractions separated by immunomagnetic sorting, we repeated the

experiments with FACS-sorted T cell fractions in one MDH patient (32) and in one monoallergic patient (491) and observed the same response pattern (Fig. 5).

Surface marker expression on the different CD4⁺ T cell subfractions

The different CD4⁺ T cell subpopulations were analyzed for their expression of activation marker CD38 and cell-exhaus-

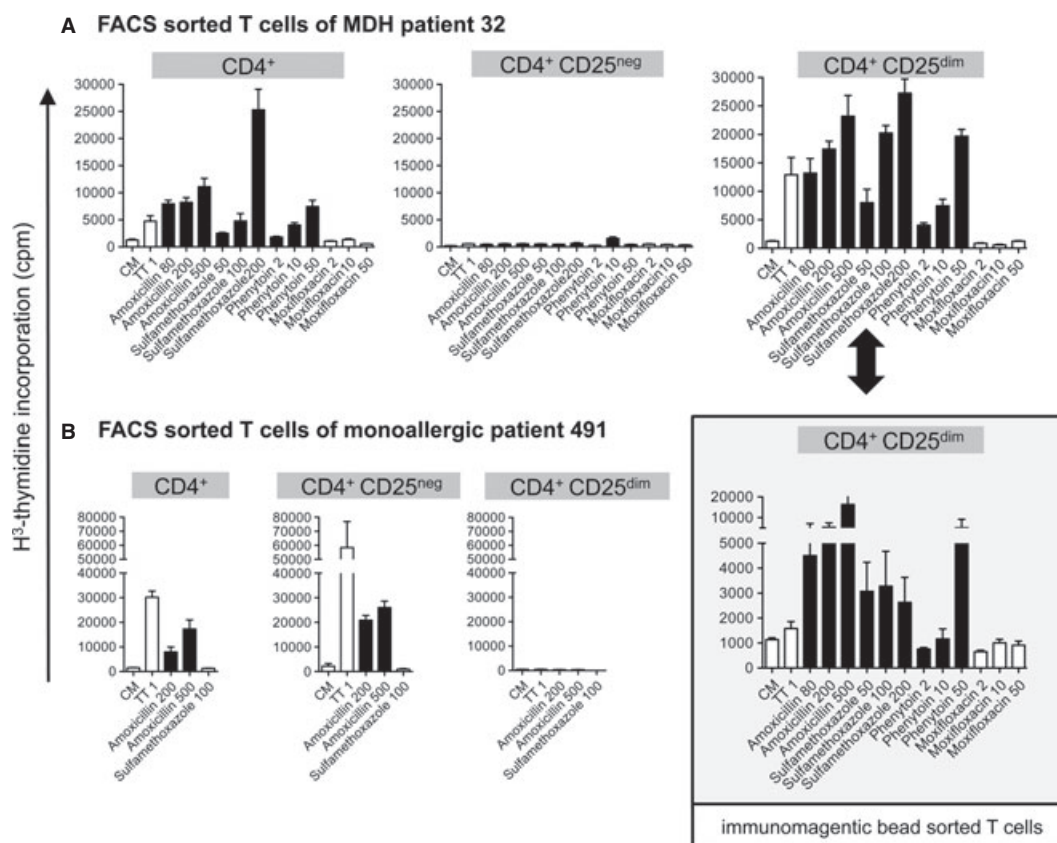


Figure 5 FACS-sorted T cell fractions show the same response pattern in one monoallergic patient and one multiple drug hypersensitivities (MDH) patient. Peripheral blood mononuclear cells were isolated from MDH patient 32 (A) and monoallergic patient 491 (B) and sorted by flow cytometry, and the same assays were performed as mentioned in Fig. 4 and Fig. 5. The drug-specific T cells were found in the same T cell compartment as shown by magnetic

bead-sorted T cells. For direct comparison, we have added the results of the immunomagnetic-sorted CD4⁺ CD25^{dim} T cell subfraction in the box. Culprit drugs are indicated as black bars, and uninvolved drugs and controls as white bars. CM, culture medium only; TT, tetanus toxoid (positive control). Concentrations are represented in µg/ml.

tion marker PD-1 (Fig. 6). When the expression of CD38 was analyzed, the only significant difference was found within the CD4⁺ CD25^{dim} T cell fraction between MDH patients and HC. Concerning cell-exhaustion marker PD-1, MDH patients showed a significantly enhanced expression in all T cell fractions studied with a tendency to higher levels in the CD4⁺ CD25^{dim} T cell subfraction.

Discussion

The pathogenesis of MDH is poorly understood, which makes the definition of MDH controversial. In this study, we restricted our analysis to MDH patients with a history of multiple hypersensitivity reactions and a positive LTT to at least two structurally distinct drugs. This focus on MDH patients with an underlying immune mechanism has the advantage that a common pathomechanism could be discerned.

The main difference between MDH and monoallergic patients was not a deficiency in Treg function or number, but the fact that the drug-reactive T cells were found in a CD4⁺ CD25^{dim} T cell subfraction, which showed an

enhanced state of activation (increased CD38), as well as signs of cell exhaustion (enhanced PD-1 expression).

In contrast to its original description (4), we found that MDH was not restricted to antibiotics. The syndrome often started with a severe and intense T cell mediated reaction, either a DRESS/DIHS associated with exposure to an anti-convulsant or a widespread maculopapular exanthema (MPE) to a drug such as amoxicillin. Previous studies have shown that cross-reactivity of drug-specific T cell receptors is rare and, if present, restricted to drugs of the same class (13–16). This study indicates that MDH was unlikely to be due to cross-reactivity as most patients developed clinical signs to structurally distinct drugs. The fact that we were able to generate separate non-crossreactive TCCs to the drugs involved in one MDH patient confirmed this assumption.

In contrast to the study by Takahashi et al. (11) in patients with monoallergic DRESS, our quantitative and functional analysis of Treg cells in MDH patients revealed a normal distribution and function when analyzed in clinical remission 4 months to 7 years after the acute event. However, our data do not exclude the possibility of a decreased

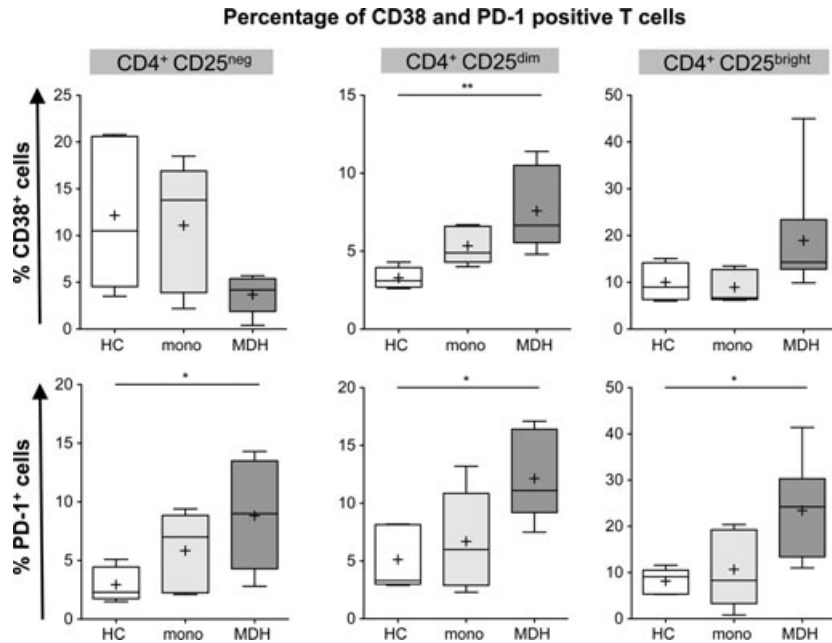


Figure 6 Other surface markers of the different CD4⁺ T cell subfractions in HC ($n = 6$), monoallergic patients ($n = 6$), and MDH patients ($n = 7$). The expression pattern of CD38 (activation) and PD-1 (cell exhaustion) was analyzed on different CD4⁺ T cell populations. CD4⁺ CD25^{dim} T cell fraction showed an enhanced expres-

sion of CD38 and of PD-1 in MDH patients compared with HC. Staining and analysis were performed on peripheral blood mononuclear cells. HC, healthy controls; mono, monoallergic patients; MDH, multiple drug hypersensitivity patients. * $P = 0.01$ – 0.05 , ** $P = 0.001$ – 0.01 .

frequency and function of Treg cells during the acute phase or immediately after resolution. All individuals tested were otherwise healthy and did not show additional protein allergies or autoimmune diseases, which may be a consequence of Treg cell deficiency (17).

T cell activation was evaluated by the upregulation of different surface markers as well as ³H-thymidine uptake. The different culprit compounds in the same individual activated both CD4⁺ and/or CD8⁺ T cells. Our functional analysis was mainly focused on the CD4⁺ T cell response, which appeared to be highly activated in our patients. However, further work is needed to analyze the CD8⁺ T cell response, which is involved in certain forms of drug hypersensitivity (18).

In healthy individuals and monoallergic patients, depletion of the CD4⁺ CD25^{bright/dim} T cell fraction enhanced the TT and the drug-specific T cell response. As expected, adding back the CD4⁺ CD25^{bright} T cells suppressed both these responses. This proves that the Treg cells were functionally intact and able to suppress T cell reactions in each patient group. The drug-reactive T cells of MDH patients were found in a different and previously undescribed T cell subfraction, namely a pre-activated CD4⁺ CD25^{dim} T cell population. Importantly, addition of CD4⁺ CD25^{bright} (Treg) T cells suppressed the drug response in MDH patients, demonstrating that the Treg cells were functionally active in MDH patients.

How can one explain the permanent and consistent activation of the drug-reactive T cell fraction in MDH patients? As the T cell activation was observed on unstimulated cells *ex vivo*, it must have already occurred *in vivo* in the absence of

drug exposure. Possible candidates for this *in vivo* activation are endogenous herpes viruses, which are known to stimulate a substantial portion of circulating CD4⁺ and CD8⁺ T cells and induce a phenotype similar to that described with the CD4⁺ CD25^{dim} cell fraction of MDH patients (19). In this respect, patients initiate their MDH with DRESS/DIHS or other severe drug reactions, which are associated with massive herpes virus reactivations (human herpesvirus-6 [HHV-6], cytomegalovirus [CMV], Epstein-Barr virus [EBV]) (20). In fact, the tissue damage (hepatitis, carditis, etc.) occurring in DRESS has recently been attributed to EBV-specific CD8⁺ T cells infiltrating the tissues (21).

In conclusion, our data show that (at least a form of) MDH can be defined by clinical and also by immunological means. A peculiar feature of MDH is the fact that the drug-reactive T cells are found in an *in vivo* pre-activated cell fraction. Further investigations are needed to identify the antigens inducing this *in vivo* T cell activation.

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Author contributions

BD, MG, TK, and WJP were involved in the study design; BD, OH, and WJP wrote significant sections of the manu-

script; BD planned and performed all T cell assays and analyzed the data; DN and KP generated and performed specificity assays; MG, DN, and OH helped to revise the manuscript; patients were recruited by BD, TW, and ML. All authors read and approved the final manuscript.

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Conflicts of interest

W. J. Pichler has consultancy arrangements with Johnson & Johnson and Pfizer, and has received grants from Pfizer Inc. The other authors have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article found at: <http://www.wileyonlinelibrary.com>

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Supplementary Table S1. **Clinical details of MDH and monoallergic patients.**

Table S 1A. MDH patient's characteristics					
Patient	Age/sex	Culprit drugs		Symptoms	Time interval*
32	68 years/m	1 st	Phenytoin	DRESS/DIHS	7,5 years
		2 nd	Amoxicillin	MPE	7,4 years
		3 rd	Sulfamethoxazole	Erythrodermia	7,2 years
303	72 years/m	1 st	Amoxicillin	Exanthema	9 years
		2 nd	Phenytoin	DRESS/DIHS	3 years
556	66 years/f	1 st	Vancomycin	DRESS/DIHS	9 months
		2 nd	Rifampicin		
		3 rd	Ceftobiprol	Agranulocytosis	10 months
553	45 years/m	1 st	Phenytoin	DRESS/DIHS	9 years
		2 nd	Carbamazepine	DRESS/DIHS	13 months
558	72 years/m	1 st 2 nd	Sulfamethoxazole Penicillin G	MPE	4 months
535	50 years/m	1 st 2 nd	Sulfamethoxazole 5-aminosalicylic acid	DRESS/DIHS	4 months
577	49 years/f	1 st	SMX	Exanthema	5 months
		2 nd	Trimethoprim	Exanthema	5 months
Table S 1B. Monoallergic patient's characteristics					
Patient	Age/sex	Culprit drug		Symptoms	Time interval*
491	54 years/m	1 st	Amoxicillin	Exanthema	13 months
47	39 years/f	1 st	Carbamazepine	DRESS/DIHS	5 years
11	47 years/m	1 st	Sulfamethoxazole	MPE, malaise	15 years
557	30 years/f	1 st	Amoxicillin	MPE	9 months
14	81 years/m	1 st	Moxifloxacin	MPE	5 years
460	51 years/f	1 st	Amoxicillin	MPE	14 months

* Time interval between acute allergy and testing.

DRESS: Drug rash with eosinophilia and systemic symptoms; DIHS: Drug-induced hypersensitivity syndrome; MPE: Maculopapular exanthema.

Supplementary Table S2. Concentrations of drugs used for all studies.

Drug	Concentrations (µg/ml)			Purchased from:
	1st	2nd	3rd	
Amoxicillin	80	200	500	Sigma, Basel, Switzerland
Carbamazepine	2	10	50	
Ciprofloxacin	0.1	1	10	
Gentamycin	1	10	100	
Penicillin G	100	200	500	
Phenytoin	2	10	50	
Propranolol	10	–	–	
Rifampicin	10	50	100	
Sulfamethoxazole	50	100	200	
Trimethoprim	10	25	50	
Vancomycin	10	50	100	
5-aminosalicylic acid	10	50	–	Bayer AG, Leverkusen, Germany
Moxifloxacin	2	10	50	
Clonazepam	2	10	50	Roche Pharma, Basel, Switzerland
Ceftobiprol	0.1	1	10	Janssen-Cilag AG, Baar, Switzerland
Iodixanol	10	–	–	GE Healthcare AG, Wädenswil, Switzerland

Supplementary Table S3. **Antibodies used for all studies.**

		Antigen	Format	Purchased from:
Activation marker	Mouse anti-human	CD3	APC	BD Biosciences, San Jose, CA, USA
		CD4	PerCP	
		CD8	PE-Cy7	
		CD25	FITC	
		HLA-DR	FITC	
		CD69	PE	
		CD71	PE	
Phenotypic characterization of T cell subsets	Mouse anti-human	CD38	FITC	Immunotech, Marseille, France
		PD-1	PE	BioLegend, San Diego, CA, USA
		CD25	PE	
		CD25	FITC	
Cell sorting	Mouse anti-human	CD4	PerCP	BD Biosciences
		CD25	PE	BioLegend
Expression of FOXP3	Mouse anti-human FOXP3 staining kit	FOXP3	FITC	eBioscience, San Jose, CA, USA
	Mouse anti-human	CD25	PE	BD Biosciences

Supplementary Table S4. **T cell clones show no cross-reactivity.** TCC were generated by limiting dilution from bulk cultures after the indicated stimulation as described in materials and methods. TCC were then tested for their proliferative response to carbamazepine, phenytoin and phenobarbital.

	Number of drug-specific TCCs			Cross-reactivity
	Carbamazepine	Phenytoin	Phenobarbital	
Carbamazepine	24	0	0	no
Phenytoin	0	2	0	no
Phenobarbital	0	0	13	no

Supplementary Figure S1. Upregulation of T cell activation markers CD69 and CD71 on CD8⁺ T cells of MDH patient 32 after drug stimulation.

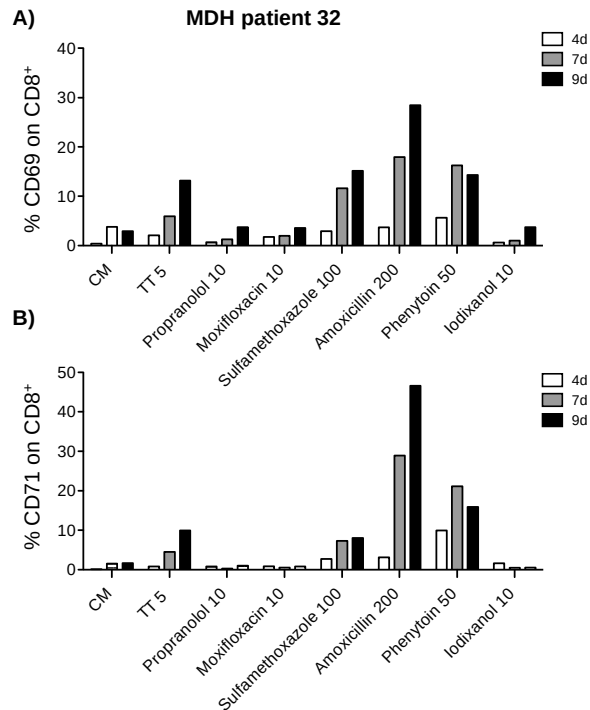


Fig. S1: Kinetics of CD69 (A) and CD71 (B) upregulation are shown at three different time points (4, 7 and 9 days). Data are representative for two further monoallergic patients tested (303 and 556). CM: culture medium only; TT: tetanus toxoid (positive control). Concentrations are shown in µg/ml.

Supplementary Figure S2. T cell isolation diagram according manufacturer's protocol

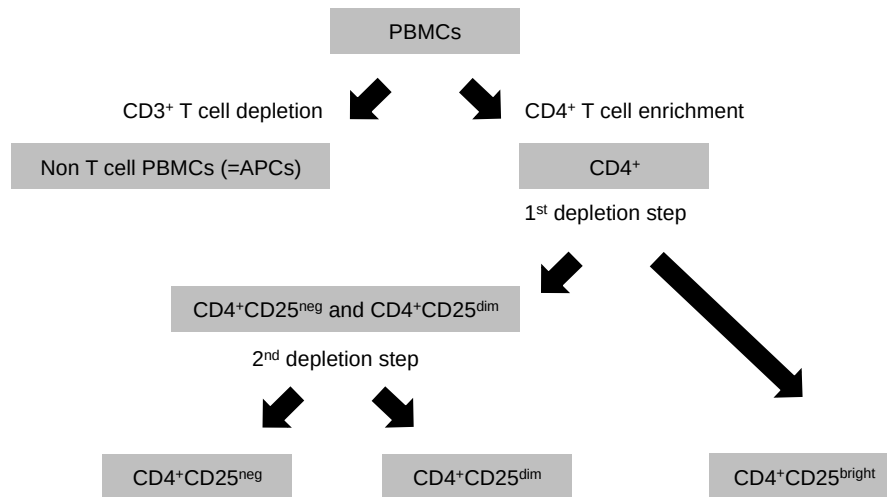


Fig. S2. The first separation step is tailored to highly enrich CD4⁺ T cells from freshly isolated PBMCs, followed by a depletion of CD4⁺CD25^{bright} T cells (1st depletion step). The supernatant contains CD4⁺CD25^{neg} and CD4⁺CD25^{dim} cells, and may be retained and subsequently depleted of all CD4⁺CD25^{dim} cells (2nd depletion step) to obtain highly purified CD4⁺CD25^{neg} (effector) T cells.

Supplementary Figure S3. **CD25⁺Foxp3⁺ expression on CD4⁺ T cells from healthy controls (n=6), monoallergic (n=6) and MDH (n=7) patients.**

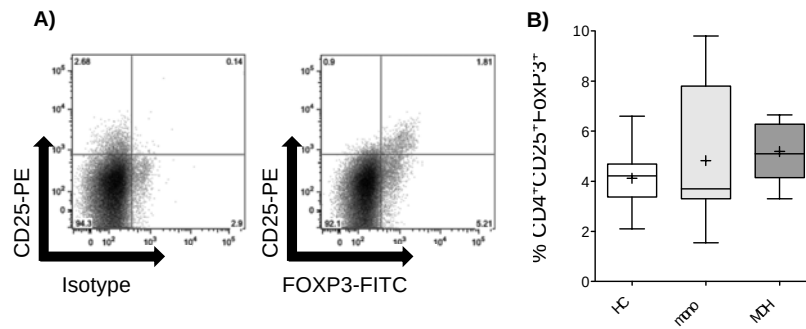


Fig. S3. A) Representative dot plot for CD25 and Foxp3 staining is shown for a monoallergic patient. Cells were gated on CD4⁺ T cells B) The relative amount of CD25⁺Foxp3⁺ T cells was determined in the CD4⁺ T cell population in all three groups. HC: healthy controls; mono: monoallergic patients; MDH: multiple drug hypersensitivity patients.