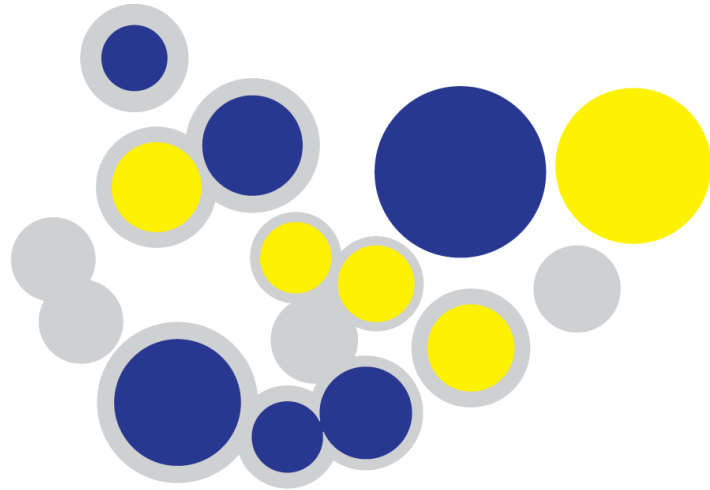


Programme



EUROBATV

Peschiera Del Garda . IT

23 – 24 September 2011

Organisers: Hans Jürgen Hoffmann, Aarhus University, Aarhus, Denmark
Bernadette Eberlein, Technical University, Munich, Germany
Cristobalina Mayorga, Carlos Haya F, IMABIS, Malaga, Spain
Edward Knol, University Medical Center Utrecht, Utrecht, The Netherlands

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EuroBAT meetings were held in

| | | |
|---------------------------------|----------------|-----------------|
| 2007 Pamplona, Spain | grass roots | 70 participants |
| 2008 St Etienne, France | grass roots | 30 participants |
| 2009 Rotterdam, Netherlands | grass roots | 66 participants |
| 2010 Munich, Germany | EAACI Endorsed | 60 participants |
| 2011 Peschiera Del Garda, Italy | EAACI meeting | 80 participants |
| 2012 (Aarhus, Denmark) | EAACI meeting | |
| 2013 | | |

PROGRAMME - OVERVIEW

Friday: EuroBAT

| | |
|---------------|---|
| 12:00 - 13:00 | Lunch and Opening (Hoffmann, Eberlein, Knol, Mayorga) |
| 13:00 – 13:30 | Ulrich Blank |
| 13:30 - 14:30 | Open presentations (Knol, Lund, Schneider,) |
| 14:30 - 15:00 | Coffee break – industrial exhibition |
| 15:00 - 15:30 | Markus Ollert |
| 15:30 - 16:30 | Open presentations (Korosec, Hoffmann, Drbal) |
| 16:30 – 17:00 | Coffee break |
| 17:00 – 18:45 | Workshops technical issues, drug-, food- and venomallergy |
| 18:45 – 19:15 | Reportback from workshops |
| 19:30 – | Dinner with ENDA |

Saturday: ENDA – EuroBAT meeting

| | |
|---------------|--|
| 09:00 – 09:30 | M^a Jose Torres |
| 09:30 – 10:30 | Open presentations (Mayorga, Çelik, Misumi) |
| 10.30 – 11:00 | Coffee break |
| 11:00 – 12:00 | Open presentations (Larenzko, Sebastian, Hasdenteufel) |
| 12:00 – 12:30 | Oliver Hausmann |
| 12:30 – 13:00 | Closing discussion & concluding remarks |



Friday, 23 September 2011

12:00 - 13:00 Light lunch and Opening of EuroBAT V

Session 1, Chair persons: Cristobalina Mayorga and Markus Ollert.

13:00 – 13:30 **Ulrich Blank**, Paris, France: Molecular mechanisms of mast cell exocytosis.

13:30 - 14:30 Abstract presentations
Knol, Edward, Utrecht, The Netherlands: Evaluating the potency of peanut allergens using washed and unwashed basophils in BAT and passively sensitized huFceRI-transduced RBL cells.
Lund, Gitte, Hørsholm, Denmark: Protocol and donor dependent activation induced scatter changes may lead to exclusion of highly activated basophils in FACS analysis.
Schneider, Michael, Schönenbuch, CH: Sources of variation in Basophil activation tests.

14:30 - 15:00 Coffee break

Session 2, Chair persons: Ulrich Blank, Bernadette Eberlein.

15:00 - 15:30 **Ollert, Markus**, Munich, Germany: Evaluation of recombinant hymenoptera venom allergens in the basophil activation test.

15:30 - 16:30 Abstract presentations
Korosec Peter, Golnik, Slovenia: Monitoring of immunotherapy with recombinant Hymenoptera venom allergens in the basophil activation test.
Hoffmann, Hans Jürgen, Aarhus, Denmark: Basophil Sensitivity Decreases in Parallel with Clinical Sensitivity in Allergen Challenge during Subcutaneous Immunotherapy (SCIT) in Subjects Allergic to Grass Pollen.
Drbal Karel, Prag, CZ: Basophil: the action hero from the scratch

16:30 – 17:00 Coffee Break

17:00 – 18:45 Workshops
Technical Issues Chair: Hoffmann
Drug allergy Chair: Mayorga
Food allergy Chair: Knol
Venom allergy Chair: Eberlein

18:45 – 19:15 Workshop feedback (Hoffmann, Mayorga, Knol, Eberlein)

19:30 - Dinner with ENDA (bus tour to an Osteria, be on time!)

Saturday, 24 September 2011

2nd ENDA- EuroBAT meeting

Session 3, Chair persons: Edward Knol and Ingrid Terrehorst.

09:00 – 09:30 **M^a Jose Torres**, Málaga, Spain: The role of betalactam determinants in the basophil activation test for the evaluation of patients with immediate hypersensitivity reactions to these drugs.

09:30 – 10:30 Open presentations

Cristobalina Mayorga, Málaga, Spain: The effect of quinolone photosensitization on the sensitivity of the basophil activation test.

Gülfem E. Çelik, Ankara, Turkey: In vitro aspirin stimulation on basophils in patients with Non Steroidal Antiinflammatory Drugs hypersensitivity.

Misumi, Denise, São Paulo, Brazil: Basophil Activation Test (BAT) in the diagnosis of hypersensitivity reactions to Nonsteroidal Antiinflammatory Drugs: two patients, two reactions to BAT.

10.30 – 11:00 Coffee break

Session 4, Chair persons: Hans Jürgen Hoffmann, and Knut Brockow.

11:00 – 12:00 Open presentations

Larenzko, Lyudmila, St.Petersburg, Russia: Detection of IgE-antibodies in dental allergy.

Sebastian, Katrin, Aachen, Germany: Evaluation of the sensitizing potential of antibiotics in vitro using the human cell lines THP-1 and MUTZ-LC and primary monocyte – derived dendritic cells.

Hasdenteufel, Frederic, Nancy, France: Usefulness of structure-activity relationships in drug anaphylaxis.

12:00 – 12:30 **Oliver Hausmann**, Bern, Switzerland: An in vitro Prausnitz-Küstner test.

12:30 – 13:00 Closing discussion & concluding remarks

How could you use BAT in drug allergy diagnosis?

How early in the diagnostic workup for drug allergy do you do BAT?

Concluding notes:

Evaluating the potency of peanut allergens using washed and unwashed basophils in BAT and passively sensitized huFceRI-transduced RBL cells

Helma van Doorn, Stans den Hartog
Jager, Andre Knulst, Els van Hoffen and
Edward Knol

Dept. Dermatology/Allergology, University
Medical Center Utrecht, Utrecht, The
Netherlands

Background: At the last EuroBAT meeting we showed that the peanut allergens Ara-h2 and Ara-h6 are superior in the activation of the basophils in our patients.

The aim of our current study was to test the potency of Ara-h1, Ara-h2, Ara-h3 and Ara-h6 in activating basophils in different experimental setups, namely whole blood basophils, washed basophils and passively-sensitized huFceRI-transduced RBL's (huRBL).

Methods: Basophil activation test was performed using both whole blood, as well as washed blood from peanut allergic donors. Basophils were stimulated with the allergens and activation was monitored by a combination of CD63, CD203C and CD123 antibodies. HuRBLs were incubated overnight with patient plasma. After removal of the plasma, huRBLs were stimulated with peanut allergens.

Results: In the whole blood BAT the $\frac{1}{2}$ max concentrations demonstrated that Ara-h6 was the most potent: 0.9+0.3 ng/ml. Washing of the basophils resulted in a decrease of the $\frac{1}{2}$ max values for all peanut allergens, but comparable $\frac{1}{2}$ max for Ara-h6 and Ara-h2. To rule out IgE independent effects of the patient basophils, we tested the potency of the peanut allergens on passively sensitized huRBLs. In this test both Ara-h2 and Ara-h6 were the most potent.

Conclusion: In our patient population both Ara-h2 and Ara-h6 are superior in the activation of the basophils. In contrast to other types of allergies, washing of basophils results in an increase in

sensitivity. The fact that Ara h 6 was only in the whole blood basophil test the most potent might be explained by a selective increase of blocking antibodies for Ara-h2 compared to Ara-h6.

Protocol and donor dependent activation induced scatter changes may lead to exclusion of highly activated basophils in FACS analysis

Gitte Lund, Jette Skovsgaard, Jens Holm
and Peter A.Würtzen
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ALK-ABELLÓ A/S, Research, Hørsholm,
Denmark

Background:The commonly used procedures for basophil activation assays (BAT) include basophil identification (gating) based on low side scatter (SSC) in combination with various markers on the basophils. We have observed activation-related changes in basophil FACS scatter profiles in whole blood as well as purified basophils. In this work we describe the exclusion of highly activated basophils from the cell population investigated in FACS experiments.

Methods: Allergen induced BAT experiments were performed with whole blood from grass allergic donors. Following 1h the expression of cell surface markers CD63 and CD203c as well as CCR3 or CD123 for identification were measured by FACS. Two protocols: staining during, or after activation and wash were compared. FACS data were evaluated with different gating strategies in an attempt to include all basophils in the analyses.

Results: Activation of basophils led to a dose-dependent change in scatter profiles coherent with increased surface expression of CD63 and CD203c in response to allergen challenge. Staining procedure had a dramatic influence on activation induced side-scatter. Scatter changes were highly variable among donors and led to up to 50% exclusion of basophils in FACS analyses using conventional basophil identification

strategies in protocols based on staining during activation. In addition it was found that CCR3 was less suitable for identifying basophils when staining after activation.

Conclusions: Highly activated basophils may be excluded from analyses in flow cytometry assays due to increased SSC, most pronounced in BAT protocols with simultaneous staining and activation. This factor has to be considered when basophil activation data is evaluated.

Sources of variation in Basophil activation tests

Schneider Michael¹, Sabine Kräuchi¹, Jermann Thomas¹

¹ BÜHLMANN Laboratories AG, Schönenbuch, CH (correspondence: ms@buhlmannlabs.ch)

Introduction

Flow Cytometry is a method that is not easy to standardise. Different technologies from different manufacturers do not make it easier. Knowledge about possible sources of variation may help to improve the quality of BAT data and helps to gain more confidence in the technology and the method.

Methods:

Apparently healthy controls (n=116) of Swiss population (age 16-65) were tested with Flow CAST[®] highsens Basophil activation test. Reference values for Basophil yield, precision, and reproducibility were established. With special interest we addressed the question: how many cells (basophils) have to be counted to get a sufficient reproducibility?

Results:

Working with 100 µl EDTA whole blood per tube the mean Basophil yield was 2395 cells (2.5th to 97.5th percentile 775 to 5840 cells) measured by Partec PAS Flow Cytometer connected to Partec RobbyWell automate. A precision profile was calculated from differently stimulated blood samples (n=20). At basophil activation above 10% the CV is below 15%. Inter Technician Variation was 6.1 - 11.7 % CV using two blood samples from

asymptomatic blood donors tested by six technicians in two different labs within the same day.

Conclusion:

At least 50 positive cells have to be counted to get a precision <15% CV. Thus a total number of at least 1000 Basophils have to be counted to be able to distinguish between low activation values. Instrument reproducibility has to be established and monitored by each lab.

Basophil Sensitivity Decreases in Parallel with Clinical Sensitivity in Allergen Challenge during Subcutaneous Immunotherapy (SCIT) in Subjects Allergic to Grass Pollen

Johannes Martin Schmid, Ronald Dahl, Hans Jürgen Hoffmann

Department of Respiratory Medicine, Aarhus University Hospital, Noerrebrogade 44, 8000 Aarhus C, Denmark

Background:

SCIT reduces the specific type-1 allergic response and is associated with significant relief of symptoms. Basophil sensitivity, defined as half maximum activation (LC50) after crosslinking of specific IgE by relevant allergen, is thought to reflect clinical intensity of allergic disease. We hypothesized that changes in basophil sensitivity are caused by both humoral factors and cellular desensitization.

Methods:

We measured changes in basophil activation in 24 subjects (18 patients on standard SCIT, 6 patients in a control group) with rhinoconjunctivitis due to grass pollen allergy. Basophil activation was measured by flow cytometry as the percentage of CD63 expression on the surface of CD193+ blood basophils activated by 8 log₁₀ dilutions of grass pollen extract (0,000256-25600 SQU/ml). This was done on washed cells and cells reconstituted with plasma from the baseline and present visit. LC50 was the primary outcome measure. At baseline and after one year of treatment, nasal and cutaneous allergen challenge was performed and participants completed a

retrospective symptom score at baseline and daily symptom scores during the pollen season.

Results:

In the treatment group, the LC50 in the samples reconstituted with present plasma changed from a mean LC50 of -0,52 at baseline to a mean of 1,64 after 15 months of treatment (n=24, p<0,001).

Looking at the cellular changes, we found a 10-fold decrease of the basophil sensitivity in the washed cells (Δ LC50: 0,98, n=16, p=0,0003), and a 10-fold decrease in the humoral component of basophil sensitivity as well (Δ LC50: 1,03, n=16, p=0,026). No significant changes were observed in the control group.

The median concentration at the positive threshold of the nasal allergen challenge increased from a median 7.943 SQU/ml at baseline to 79.433 SQU/ml after one year of treatment, while a positive skin prick test was found at a median concentration of 1.995 SQU/ml at baseline and 25.118 SQU/ml after one year in the treatment group. In the same period, the maximum symptom score decreased from 10,6 at baseline to 5,6 after one year.,No significant clinical changes were seen in the control group.

Conclusion:

In the treatment group, there was a fast development of humoral protection and a slow development of cellular desensitization. This results in a 100-fold decrease in basophil sensitivity, which is paralleled by a significant improvement of clinical outcome measures.

Monitoring of immunotherapy with recombinantHymenoptera venom allergens in the basophil activation test

Peter Korošec, Nina Čelesnik, Renato Eržen, Mira Šilar, Irene Mittermann*, Rudolf Valenta*, Mihaela Zidarn, Mitja Košnik

University Clinic Golnik; *Medical University of Vienna

Introduction:

New *in vitro* methods are essential for developing better follow-up criteria for venom immunotherapy (VIT).

Methods:

We included 10 wasp and 8 honeybee venom allergic subjects. Basophile CD63 activation test (BAT) was performed with major recombinant Hymenoptera venom allergens rApi m1, rApi m2 and rVes v5, just before and 6 months after VIT starting.

Results:

All 10 wasp venom allergic subjects showed positive BAT response to rVes v5 and negative to rApi m1. One subject showed also positive response to rApi m2. Of 8 honeybee venom allergic subjects, 7 showed positive responses to rApi m1, 5 of them were also positive for rApi m2, and one subject was positive only for rApi m2. None of them was positive for rVes v5. After 6 months of immunotherapy BAT response to rVes v5 decrease in 3 of 10 wasp allergic subjects, in one subject there was also a decrease in rApi m2. In honeybee venom allergic subjects the rApi m1 decrease was evident in all 7 subjects, and 5 of them also showed a decrease in rApi m2. However, there was no decrease in a subject positive only for rApi m2. The recombinant based specific decrease was mostly evident as a shifting of CD63 dose-dependend curve to the right. No additional VIT related sensitization was evident for cross reactive rApi m2 (hyaluronidase) in any of study subjects.

Conclusions:

VIT performed with whole allergen extract induce component specific changes in basophil allergen response. This dynamic seems to be different for a wasp and honeybee venom allergy. Further, mechanism orientated and more long term studies are needed.

Title: Autoimmune-allergies to hormones.

Authors: **Shilpa Shah***, Roby Russell**, Patricia Richardson***, Richard Richardson***.

Institutions: *University of Mumbai, India, **Roby Institute, Austin, Texas, USA, ***University of Texas at Austin, Texas, USA.

All hormones can act as allergens. Different derivatives of estradiol and progesterone bound to serum proteins such as bovine serum albumin (BSA), e.g., β -estradiol-6-carboxymethyl-BSA, β -estradiol-6-carboxymethyl-BSA, progesterone-11- α -BSA, progesterone-7-BSA, progesterone-3- carboxymethyl-BSA etc. are commercially available. These protein-conjugated hormones are injected into animals, to prepare monoclonal and polyclonal antibodies against them. The ease with which these antibodies are formed is strong evidence for the immunogenicity of oestrogen and progesterone after their binding to carrier proteins. 'Autoimmune-allergy to hormones' is an allergic reaction where the offending allergens are hormones. It is an immune reaction to the hormones, which can interfere with the normal function of the hormones. It occurs in almost all women during the perimenstrual part of their cycle. In some women it gets so pronounced that it results in an actual disease. The perimenstrual allergies are about the cyclic abundance of the hormone causing a cyclic expression of allergic symptoms. Because hormones are internal antigens, these conditions could also be described as "hormone autoimmunity" but due to variations in the levels of hormones in human body with time (just like that with external allergens) and due to appearance and disappearance of symptoms (just like that with allergy) and also due to presence of various classes of hormone specific antibodies including IgE, these conditions are rightly described as autoimmune-allergies to hormones. It can cause disease conditions like anxiety and panic attacks, premenstrual syndrome, premenstrual asthma, menstrual migraine, fibromyalgia, interstitial cystitis. It could be a plausible cause of idiopathic urticaria, unexplained arthritis, chronic fatigue

syndrome and unexplained infertility. Autoimmune-allergies to hormones can be diagnosed and treated in a routine allergy clinic.

Basophil: the action hero from the scratch

Drbal Karel, ExBio, Prag, CZ.

Basophils stand both at the starting point of the allergic response as well as drive the final effector phase. These cells have the potential to sense the enemies, present them to the immune system plus mobilize all deadly weapons to combat them at the end. I will compare the current knowledge on basophil function in mouse models to the human disease with regard to Ag-presenting capacity, IgE/IgG ratios and alternative anaphylaxis, aggregated / monomeric Ig, acute / chronic response, specific receptor expression and another functions such as B cell activation and memory.

Abstracts: ENDA-EuroBAT meeting

The effect of quinolone photosensitization on the sensitivity of the basophil activation test

Cristobalina Mayorga

Research Laboratory, Carlos Haya-F
IMABIS, Malaga, Spain

In the last decade, there has been an increase in the number of reported immediate hypersensitivity reactions induced by fluoroquinolones, being urticaria and anaphylaxis the most frequently described. These observations, especially the occurrence of more severe reactions, such as anaphylaxis and anaphylactic shock, have been associated with the introduction of moxifloxacin for therapeutics.

Basophil activation test (BAT) can be useful for evaluating immediate hypersensitivity to fluoroquinolones. Previous evidence indicate that comparisons of the capacity of activation of basophil with ciprofloxacin and moxifloxacin in cases very well validated, the response to ciprofloxacin appear in higher proportion compared with moxifloxacin, even in those cases where moxifloxacin was the culprit drug. As fluoroquinolones are photoreactive, whether laboratory light exposure can influence in the induction of photoproducts and therefore modify BAT results is unknown. For this it is important to analyze the effect of laboratory light in ciprofloxacin and moxifloxacin degradation, drug-protein conjugate formation and therefore in basophil activation in patients with immediate hypersensitivity reactions to fluoroquinolones.

The study of patients with confirmed immediate hypersensitivity to fluorquinolones (ciprofloxacin or moxifloxacin) and quinolones tolerant controls with BAT was done in laboratory light and dark conditions. The fluoroquinolones degradation absorption and emission measurements were performed in aqueous solution and in whole blood. In the latter supernatant was divided in high (>3000Da) and low (<3000Da) molecular weight fractions before analysis. The results showed that BAT positivity was higher in dark

conditions (57.1%) than in light (46.4%) mainly due to an increase in moxifloxacin results from 17.9% to 35.7% with no changes in ciprofloxacin results (46.4% in both conditions). There was an important decrease in the fluorescence emission intensity under light conditions for high and low molecular weight fractions for moxifloxacin without changes for ciprofloxacin.

These indicate that BAT response in patients with immediate hypersensitivity depend on the photolability of the fluoroquinolone tested. In fact in laboratory light conditions moxifloxacin was degraded producing a lower amount of both free (<3000Da) and fluoroquinolone-protein conjugates (>3000Da). This suggests that laboratory light conditions induce photodegradation of fluoroquinolones, especially moxifloxacin, therefore influencing the basophil activation results. This is important when evaluating immediate hypersensitivity to fluoroquinolones.

Gülfem E. Çelik, Ankara, Turkey: In vitro aspirin stimulation on basophils in patients with Non steroidal antiinflammatory drugs hypersensitivity

Basophil Activation Test(BAT) in the Diagnosis of Hypersensitivity Reactions to nonsteroidal antiinflammatory drugs: two patients two ractions to BAT

Misumi, DS; Ensina, LF; Tanno, LK; Motta, AA; Sousa, LB; Kalil, J; Kokron, CM.

Laboratory of Clinical Immunology and Allergy (LIM-60), School of Medicine, USP - São Paulo - Brazil

Background: Reactions upon exposure to NSAIDs comprises more than 60% of patients' complains in the Adverse Drug Reactions (ADR) Outpatient Clinic of Hospital das Clínicas, São Paulo. Diagnosis to this type of hypersensitivity relies on patients' history. Although drug provocation tests (DPT) are considered the gold standard, these tests are carried out just in selected cases due to potential risks for patients, including anaphylaxis.

For more than ten years, Basophil Activation Test (BAT) has been widely studied, especially in the Allergy field. In some cases, it seems a promising diagnostic tool. Thus, we decided to test BAT to NSAID hypersensitive patients from ADR Clinic, as a possible additional tool in the diagnosis.

Objective: Compare two NSAID hypersensitive patients who had different results to BAT.

Methodology: Both patients reported urticaria and angioedema after ingestion of NSAIDs. Patient 1, whose clinical history suggested reactions to dipyron and paracetamol, had a positive oral provocation test with 50 mg of paracetamol. Patient 2 presented a history suggestive of hypersensitivity to dipyron, diclofenac and paracetamol, with positive oral provocation test to the last drug. Whole blood from both patients were incubated with different concentrations of paracetamol and dipyron. Then, labeled with the monoclonal antibodies: CD45-PercP, IgE-FITC and CD63-PE. Finally, samples were read by FACSCanto flow cytometer.

Results: Patient 1 had positive BAT to paracetamol, with the more diluted concentrations, and negative BAT to dipyron. Patient 2 had no positive result to BAT.

Conclusion: Patients may respond differently to BAT, regardless their clinical characteristics.

Detection of IgE - antibodies in dental allergy

Lyudmila Lazarenko

Federal State Organization North-Western Regional District Medical Center, Department of Allergy and clinical immunology, Saint-Petersburg, Russian Federation

Background: Allergy to local anaesthetics and dental materials are very common, but immune mechanism of this reactions is not quite clear. The aim of the study was to determine the diagnostic value of detection IgE- antibodies in cases of

dental allergy.

Method: 55 outpatients with symptoms of hypersensitivity (HS) to local anaesthetics (LA) - Group A and 317 outpatients with hypersensitivity to dental materials (DM) - Group B were investigated for specific IgE-antibodies in serum. Assay were arranged by ELISA method, Bio-Tek Instruments, reagents DR FOOKE (Germany). We studied patients with controversial results of patients case history and prick (Group A) or patch (Group B) skin tests. No drug provocation tests were done because of risk of immediate allergic reactions with a positive history of multi hypersensitivity. Group A (n=55) consist of 41 females, 14 males, mean age 38,4 years(6-80), group B (n=317) - of 229 females and 88 males, mean age 54 years (31-82). For in vitro specific IgE EAST we used such local anaesthetics allergens as :group "caines" - ultracaine, benzocaine, articaine, tetracaine; group "amides"- mepivacain, bupivacain, prilocain, lidocain; dental materials allergens - acrylon, gold, platinum, palladium, cuprum, cobalt, nickel, chromium.

Result: We found positive IgE levels to LA in 13 causes (14.8%). In the second group, sensitive to dental materials (group B), IgE-specific antibodies were positive to acrylon in 17.4 % causes. Allergy for metals were investigated for: cuprum - 25.4 %, cobalt - 31.8%, nickel - 33.3%, palladium - 21.1%, chromium - 28.3%, gold - 23.1%, platinum - 27.3% of patients.

Conclusion: Analysis of drug HS in dentistry by means of ELISA testing IgE - antibodies in serum of allergic patients proved a useful and reliable approach for an in vitro detection and characterization of drug HS. IgE-mediated allergy to LA is rare; it is more " myth, than reality". Allergy to DM is more frequent in compare with LA. This results suggest that HS in dentistry may conclude not IgE-mediated reactions and other investigations may be delivered in order to dissolve this problem.

Evaluation of the sensitizing potential of antibiotics in vitro using the human cell lines THP-1 and MUTZ-LC and primary monocyte – derived dendritic cells

Katrin Sebastian; Hagen Ott, M.D.; Gabriele Zwadlo-Klarwasser, Ph.D.; Katharina Czaja; Jens Malte Baron, M.D.; Hans F. Merk, M.D.

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Hypersensitivity reactions to beta-lactams and sulphonamides continue to be the most frequent cause of drug reactions. Recently, the European Network for Drug Allergy (ENDA)/European Academy of Allergy and Clinical Immunology (EAACI) has standardized the diagnostic procedure for allergic reactions to β -lactams. There is an urgent need for the development of alternative in vitro test systems to replace in vivo allergy testing. Therefore, we investigated the role of β -lactams and sulphonamides in delayed – type hypersensitivity (DTH) reaction by evaluating the sensitization potential in vitro using the human cell lines THP-1 and MUTZ-LC as well as primary monocyte – derived dendritic cells (moDCs). The differential gene expression of marker genes related to immunological reactions (IL-8 and IL-1 β) and to oxidative or metabolic stress responses (CES1, NQO1 and GCLM), as well as of genes encoding for transcription factors (PIR and TRIM16) was examined by real-time PCR analysis. The β -lactam antibiotics benzylpenicillin (PenG), phenoxymethylpenicillin (PenV) and ampicillin, as well as the sulphonamide sulfamethoxazole (SMX) were selected for experiments among the contact sensitizer TNBS and the irritant SDS. PenG and PenV strongly induced the expression of the marker genes NQO1, GCLM, PIR, TRIM16 and IL- β both in THP-1 cells and in moDCs, whereas ampicillin did weak to moderate or even not. Interestingly, in THP-1 cells, IL-8 was

neither stimulated by the contact sensitizer TNBS nor with the antibiotics studied, in contrast to moDCs, where the stimulatory effect of the drugs was also found on protein level. Furthermore, TNBS did not change IL-1 β gene expression in THP-1 cells, but PenG and PenV did. However, the prohaptens SMX did not change marker gene expression both in THP-1 cells and in moDCs, even though PIR is slightly upregulated in THP-1 cells and CES1 and PIR are slightly upregulated in moDCs. Notably, MUTZ-langerhans-like cells are not suitable for detecting the sensitization potential of antibiotics in vitro. In conclusion, we could demonstrate that the evaluation of the sensitization process of β -lactam antibiotics in vitro is dependent on the used cell system and marker genes studied and that different cell reactions are involved in the DTH reaction of antibiotics. Furthermore, the sensitization potential of prohaptens can not be sufficiently detected in vitro, which indicates that more attention has to be given to their metabolism.

Usefulness of structure-activity relationships in drug anaphylaxis

Frédéric Hasdenteufel, Samuel Luyasu, Nicolas Hougardy, Paul-Michel Mertes and Gisèle Kannya

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Department of Internal Medicine and Intensive Care, Clinic of South Luxembourg, Arlon, Belgium.

Department of Biology, Clinic of South Luxembourg, Arlon, Belgium.

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Structure-activity relationships (SARs) refer to the relation between chemical structure and pharmacologic activity for a series of compounds. They have been extensively used in the pharmaceutical, chemical and cosmetic industries, especially for drug and chemical design purposes, and they have also proven useful for risk assessment in toxicology and environmental science.

After succinctly presenting the SAR concept, the potential interest of SAR-based approaches in drug anaphylaxis assessment will be discussed in the light of two selected examples: betalactam antibiotics¹ and magnetic resonance imaging contrast agents². In each study, drug chemical structures were related to either skin or biological reactivity; if needed, challenge tests were carried out with an alternative drug belonging to the same therapeutic class.

In conclusion, SARs can prove useful to (i) predict the allergenic potential of a chemical or a drug, (ii) help identify putative antigenic determinants for each patient or small group of patients sharing the same cross-reactivity pattern, and (iii) predict the likelihood of adverse reactions to related molecules and select safe alternatives. In our opinion, simple, qualitative SARs should be implemented in current allergological practice through synergies between clinicians and pharmacists.

PARTICIPANTS EuroBAT V

| Name | Surname | City | Country | Comments |
|-----------------|----------------|---------------|----------------|---------------------|
| Francesca | Antonini | Genova | Italy | |
| Paul | Baars | Amsterdam | Netherlands | |
| Ulrich | Blank | Paris | France | Invited Speaker |
| Danijela | Bokanovic | Graz | Austria | |
| Antonella | Casalaro | Genova | Italy | |
| Nina | Celesnik | Golnik | Slovenia | |
| Pietro Luigi | Di Guiseppe | Acireale | Italy | |
| Karel | Drbal | Prague | Czech Republic | ExBio, speaker |
| Bernadette | Eberlein | Munich | Germany | Organiser |
| Luis | Ensina | Sao Paulo | Brazil | |
| Ernesto | Ferreira | PoA | Brazil | |
| Pierre | Gumowski | Meyrin | Switzerland | |
| Oliver | Hausmann | Bern | Switzerland | |
| Hans Jürgen | Hoffmann | Aarhus | Denmark | Organiser, speaker |
| Grazyna | JasieniakPinis | Krakow | Poland | |
| Martin | Kase | Prague | Czech Republic | ExBio |
| Edward | Knol | Utrecht | Netherlands | Organiser, speaker |
| Stergios | Kogas | Athens | Greece | |
| Peter | Korosec | Golnik | Slovenia | Speaker |
| Lilian | Krischan | Munich | Germany | Technical help |
| Petr | Kucera | Prague | Czech Republic | ExBio |
| Magdalena | Kujawiak | Lodz | Poland | |
| Lyudmila | Lazarenko | St Petersburg | Russia | speaker |
| Leonora | Lleshi | Gjakova | Kosovo | |
| Gitte | Lund | Hørsholm | Denmark | ALK Abello, speaker |
| Cristobalina | Mayorga | Malaga | Spain | Organiser |
| Jose | Motta | | Brazil | |
| Marie-Christine | Müller | Schönenbuch | Switzerland | Bühlmann |
| Markus | Ollert | Munich | Germany | Invited Speaker |
| Ilaria | Puxeddu | Pisa | Italy | |
| Michele | Romano | Schönenbuch | Switzerland | Bühlmann |
| Alexandra | Santos | London | UK | |
| Michael | Schneider | Schönenbuch | Switzerland | Bühlmann, speaker |
| Beatriz | Sevilla-Jensen | Aarhus | Denmark | Technical help |
| Shah | Shilpa | Mumbai | India | speaker |
| Denise | ShimboMisu | Sao Paulo | Brazil | speaker |
| Mira | Silar | Golnik | Slovenia | |
| Markus | Steiner | Aslzburg | Aurtria | |
| Jose | Torres | Malaga | Spain | |
| Tina | Vesel | Ljubljana | Slovenia | |
| Jolana | Vosahlova | Prague | Czech Republic | ExBio |
| Ye | Young Min | | Korea | |
| Monica | Zamfirescu | Bukarest | Romania | |
| Giovanna | Zanoni | Verona | Italy | |