BŐRGYÓGYÁSZATI ÉS VENEROLÓGIAI SZEMLE • 2015 • 91. ÉVF. 4. 135–139. • DOI 10.7188/bvsz.2015.91.4.1.

A természetes regulatív T sejtek szerepe atópiás dermatitisben

The role of natural regulatory T cells in atopic dermatitis

GÁSPÁR KRISZTIÁN DR.[1], BARÁTH SÁNDOR DR.[2], KAPITÁNY ANIKÓ DR.[1], SZEGEDI ANDREA DR.[1]

Debreceni Egyetem, Általános Orvostudományi Kar, Bőrgyógyászati Tanszék, Bőrgyógyászati Allergológiai Tanszék[1]
Debreceni Egyetem, Általános Orvostudományi Kar, Labormedicina Intézet[2]

ÖSSZEFOGLALÁS

Az atópiás dermatitis (AD) egy krónikus, remissziókat és relapszusokat mutató, kifejezett viszketéssel járó, nem fertőző, gyulladásos bőrbetegség. A növekvő ismeretanyag segít megérteni a betegség kialakulásának hátterében álló, klinikailag is releváns patofiziológiai eseményeket. A szerzők bemutatják a természetes regulatív T (nTreg) sejteket, és az AD kialakulásának hátterében álló immunológiai folyamatokban játszott szerepüket. Az nTreg sejtek számának változásai fontos indikátorai a betegség súlyosságának. Emellett a sejtek funkciójának zavara is megfigyelhető az atópiás mikrokörnyezet hatására.

Kulcsszavak:
atópiás dermatitis - regulatív T sejtek - *Staphylococcus* enterotoxin B

SUMMARY

Atopic dermatitis (AD) is a chronic, non-contagious, pruritic inflammatory skin disease showing frequent remissions and relapses. The expanding knowledge about the disease helps to understand the clinically relevant pathophysiological processes behind the development of the disorder. The authors present the natural regulatory T (nTreg) cells and their role in the immunological alterations in AD. The changes in the number of nTreg cells are important indicators of the severity of the disease. Besides the functional impairment of nTreg cells is apparent in the atopic microenvironment.

Key words:
atopic dermatitis - regulatory T cells - *Staphylococcus* enterotoxin B

Az atópiás dermatitis (AD) egy gyakori, krónikus lefolyást mutató, remissziókkal és fellángolásokkal kísért gyulladásos bőrbetegség, melyet a bőr szárazsága és kifejezett viszketése jellemez.

Számos vizsgálat eredménye segítette a betegség patomechanizmusának megértését, de a pontos részletek máig sem tisztázottak. Az AD egy multifaktoriális betegség, hátterében genetikai prediszpozíció talaján a fiziko-kémiai bőrbarrier sérülése, valamint a természetes és szerzett immunválasz zavara (beleértve az immuntolerancia eltéréseit) játszik szerepet, ahol a tünetek kialakulását különböző környezeti faktorok (pl. allergiás szenzitizáció és mikrobiális elemek jelenléte) is súlyosbíthatják (1, 2).

Az immunválasz szabályozásában fontos szereplők a regulatív tulajdonsággal rendelkező T sejtek. Az egyik ilyen sejtcsoport a forkhead box P3-t (FOXP3) expresszáló $CD4^{+}CD25^{bright}FOXP3^{+}$ természetes (natural) regulatív T (nTreg) sejtek csoportja, melyek szerepe intenzív kutatások tárgya AD-ben. Ugyanakkor az nTreg funkciók jellemzőire vonatkozó vizsgálati eredmények ellentmondásosak a betegségben. Cikkünk az nTreg sejteket és az AD kialakulásában betöltött szerepüket mutatja be.

Az adaptív immunmechanizmusok változásai AD-ben

Az AD immunmechanizmusainak megértéséhez elengedhetetlen a szerzett immunválasz résztvevőinek, közülük is elsősorban a T sejteknek az ismerete. A T lymphocyták központi szerepet játszanak a betegség lefolyásában. A bőrt infiltráló T sejtek között nagy számban találjuk a bőr (cutan) lymphocyta-asszociált antigén $(CLA)^{+}$ memória és effektor T sejteket, melyekre a betegség akut fázisában a bőrben a barrier defektus következtében fenntartott T helper 2-típusú (Th2) citokin termelés (interleukin(IL)-4, IL-5, IL-13) jellemző (3). Megfigyelhetők azok az egymásra kölcsönösen ható és egymást erősítő események, miszerint a Th2 túlsúlyú gyulladás elősegíti a barrier károsodását, illetve a sérült barrieren keresztül átjutó kifejezett allergén

Levelező szerző: Dr. Gáspár Krisztián
e-mail: nurk7@freemail.hu

expozíció Th2 túlsúlyt idéz elő. Az AD krónikus fázisa során a bőrben már nem csupán Th2 sejtek detektálhatók, hanem a Th2 sejtek mellett fontos szerepet játszanak a Th1 és Th22 sejtek is (1, 4). A vérben a bifázisos Th eloszlás a bőrrel ellentétben nem jellemző, itt Th2 túlsúly figyelhető meg a betegség akut és krónikus szakaszában is.

Az immunológiai barrier AD-ben lejátszódó változásainak részletes megismerése napjainkban új terápiák kifejlesztéséhez vezetett. Ilyen, jelenleg III. fázisú klinikai vizsgálatokban tesztelt biológiai terápiás kezelés az anti-IL-4R ellenes antitest (dupilumab), mely mind az IL-4, mind az IL-13 hatását blokkolja, ezáltal gyors és jelentős javulást hozva a súlyos klinikai tüneteket mutató, terápiarefrakter AD betegeknek (5).

A természetes regulatív T sejtek

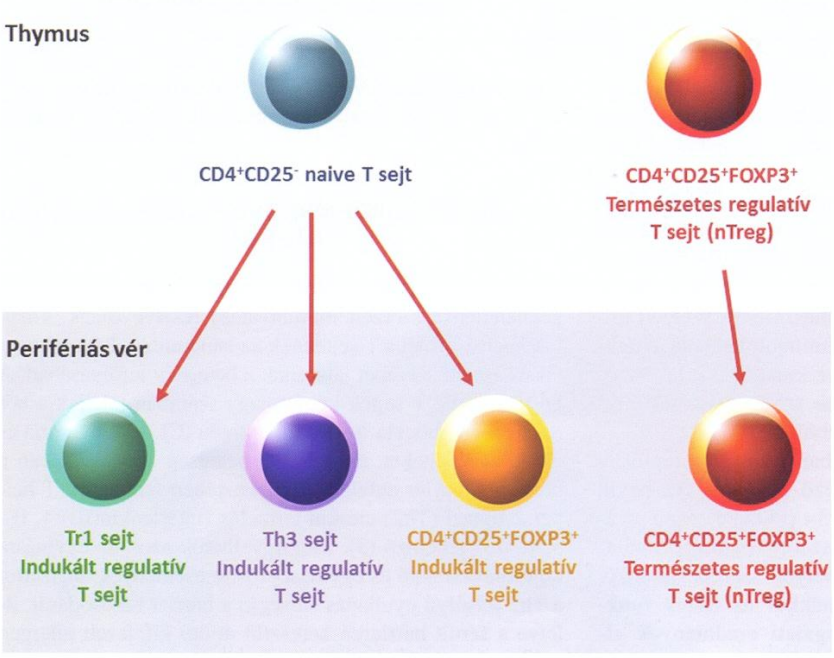
A jól működő immunrendszer alapvető feladata, hogy felismerje a saját és az idegen antigéneket, megkülönböztesse az ártalmas és az ártalmatlan behatásokat, és ezek alapján kontrollálja az immunválaszokat (a káros struktúrák ellen védekezzen a szervezet, míg a saját struktúrákat tolerálnia kell). A patológiás immunreakciók akár immundeficienciához, akár túlzott immunválaszhoz is vezethetnek. A folyamatok szabályozásában van szerepe a Treg sejteknek is, melyek a perifériás immuntolerancia fontos elemei. Feladataik közé tartozik az autoreaktív sejtek működésének szuppresszálása, az immunológiai tolerancia kialakítása (6).

A Treg sejtek egészséges felnőttekben a $CD4^+$ sejtek 5-10%-át teszik ki. A sejteknek 3 fő csoportja ismert (kialakulásukat az *1. ábra* mutatja be): a transzformáló növekedési faktor-β (transforming growth factor-β) termelő TGF-β^+FOXP3^- Th3 sejtek, az IL-10 termelő IL-10^+ $FOXP3^-$ immunszuppresszív Tr1 sejtek, valamint a $CD4^+CD25^{bright}$ $FOXP3^+$ Treg sejtek (7, 8). Ez utóbbi nTreg sejtek részt vesznek az allergénspecifikus immunválasz, így a normál immunhomeosztázis szabályozásában és az antigén prezentáló sejtek, effektor T sejtek és hízósejtek gátlásában is (9-11). Gátolják továbbá a T sejtek differenciációját, aktivációját, proliferációját, citokin szekrécióját, migrációját is. Ezen túlmenően az aktív nTreg-ek feladata az autoaggresszív lymphoproliferatív betegségek kialakulásának megelőzése, az immunválasz modulálása infekciók, autoimmun folyamatok, malignus betegségek kialakulása során (12).

Az nTreg sejtek létrejötte a thymusban kétlépcsős folyamat eredménye. Először a T sejt receptor szignál CD25 upregulációt okoz, létrehozva az nTreg prekurzorokat, majd az IL-2 szignál a FOXP3 expressziójához vezet (6). A humán nTreg sejtek a CD25 antigént fokozott mértékben expresszálják ($CD25^{bright}$) szemben a $CD25^{low}$ sejtekkel, és szuppresszív tulajdonságokkal csak a $CD25^{bright}$ sejtek rendelkeznek. A $CD25^+$ sejtek tehát nem kizárólag regulatív sejtek, közöttük lehetnek effektor funkciókkal bíró sejtek is.

A regulatív funkció kimutatása ugyanakkor igen nagy technikai kihívás, mert nincs olyan kizárólagos és specifikus marker az nTreg sejteken, amely akár fenotípusukat, akár funkciójukat vagy aktivitásuk mértékét tükrözné. A regulatív T sejtek azonosítására a legelterjedtebb módszer az áramlási citometria. Napjainkban a fent említett CD4 és CD25 markerek mellett a Treg sejtek azonosításához használt, leginkább elfogadott molekula a FOXP3 transzkripciós faktor jelenlétének, továbbá a CD127 sejtfelszíni molekula alacsony expressziójának detektálása. Pontosabb meghatározást tehet lehetővé a FOXP3 lókusz demetiláltságának kimutatása. Ez a módszer identifikálhatja a szuppresszor funkcióval rendelkező sejteket (13), habár emberben a FOXP3 önmagában kevésbé meghatározó a regulatív tulajdonsággal bíró sejtek kimutatásában (6).

Ismert tulajdonság a T sejtek plaszticitása is, azaz nem minden T sejt alcsoport végérvényesen differenciált, hanem a sejtek plasztikusak, és megváltozott gyulladásos milieu-ben a mikrokörnyezettől függően képesek a memória T sejtek különféle fenotípust és funkciót „felvenni" (14). Így az nTreg sejtek képesek akár Th2 sejtté alakulni a FOXP3 expresszió csökkenése által (15). A folyamat fordítva is végbemehet, valamint egyéb effektor T sejtek



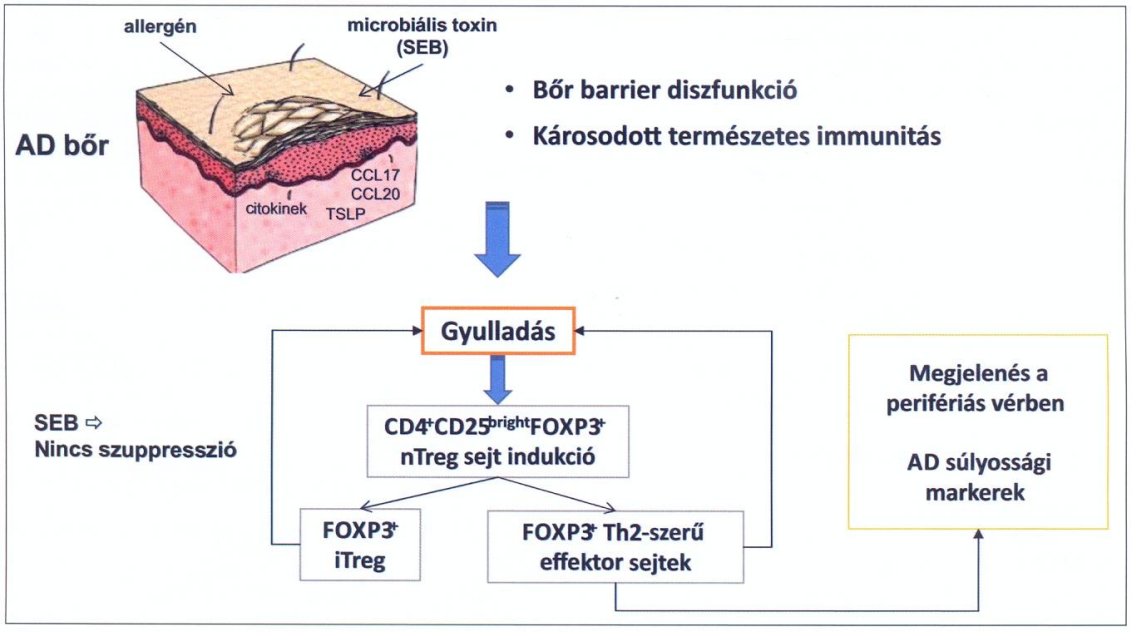
1. ábra
A $CD4^+$ regulatív tulajdonsággal rendelkező T (Treg) sejtek formái

irányába is lejátszódhat. A folyamatban központi szerepet játszik a TGF-β. Az nTreg sejtek kialakulása 3 fő mechanizmus szerint történhet. A sejtek nagy része a thymusban alakul ki („thymus" nTreg-ek). Ezek a $FOXP3^+$ sejtek már érésük során megszerzik regulatív irányú elkötelezettségüket, de emellett – például mikrobiális antigéneket bemutató $CD103^+$ dendritikus sejtek hatására – a periférián is végbemehet az nTreg-ek kialakulása („perifériás" nTreg-ek) (16), továbbá a hagyományos $CD4^+$ Th sejtek TGF-β jelenlétében a T sejt receptor stimuláció hatására „indukált" Treg-eket hozhatnak létre *in vitro*, mely folyamat az effektor T sejtek citokin indukált differenciációjához hasonlóan történik. Az így létrejött differenciált Treg-ek a fenotípusban hasonló T effektor sejteket regulálják (12, 17).

Az nTreg sejtek szerepe AD-ben

A Treg-ek emberben fiziológiás körülmények között nemcsak a perifériás vérben és a nyirokszervekben fordulnak elő, hanem számos olyan struktúrát hordoznak felszínükön, melyek a bőrbe való vándorlásukat segítik elő, ezáltal mintegy sugallva szerepüket az immunszabályozásban bőrgyulladás során. Ugyanakkor mégis kevés ismerettel rendelkezünk az nTreg sejtek szerepéről és funkciójáról atópiás bőrben. Érdekes megfigyelés, hogy a *FOXP3* gén mutációja esetén nem keletkeznek nTreg-ek, és hiányukban immundiszreguláció, polyendokrinopathia, enteropathia, X-hez kötött szindróma alakul ki, ahol AD-szerű tünetek (súlyos eczema, eosinophilia, emelkedett immunglobulin (Ig)E szint, allergiás légúti betegségek, ételallergia) is megjelennek (18). Ezen betegség megfigyelése vezetett ahhoz a kérdéshez, hogy vajon az nTreg-ek számbeli és/vagy funkcióbeli változásai szerepet játszanak-e az AD kialakulásában (6, 8, 19)?

Kevés és ellentmondásos eredményeket bemutató humán vizsgálat történt az nTreg szám és funkció meghatározására AD-ben. Tejfehérje allergiásokat vizsgálva a perifériás vérben nem találtak eltérést az nTreg számban és funkcióban, ugyanakkor atópiás hajlamot mutató betegekben a pollenszezon idején alacsonyabb allergén-specifikus nTreg számot mértek a vérben (20). A felnőtt AD populációban számos vizsgálat a keringő $CD4^+CD25^{bright}$ $FOXP3^+$ nTreg sejtek magasabb számát írta le és azok pozitív korrelációját a betegség súlyosságával, továbbá feltételezték, hogy ezek a sejtek nem működnek megfelelően (6, 21-23). Más kutatócsoportok azonban az egészséges donorpopulációban talált nTreg számmal megegyező mennyiségű sejtet találtak AD betegek perifériás vérében (24). Saját vizsgálatainkban a perifériás vérben karakterizáltuk az nTreg-ek, ezen belül a speciális, bőrbe érkező nTreg sejtek komplex mennyiségi és funkcionális változásait AD betegekben (25). Azt találtuk, hogy a regulatív funkcióval rendelkező nTreg és a bőrbe vándorló CLA^+ nTreg sejtpopulációk százalékos előfordulása szignifikánsan emelkedett volt AD betegek perifériás vérében az



2. ábra

A $CD4^+CD25^{bright}FOXP3^+$ nTreg sejtek feltételezett szerepe AD-ben. Az atópiás bőrfelszínen lévő allergén és bakteriális stimulusok gyulladáshoz vezetnek, mely a $FOXP3^+$ T sejtek helyi indukcióját okozza. Ezek a sejtek lehetnek regulatív funkcióval rendelkező indukált Treg-ek, melyek funkcióját a folyamatos SEB jelenlét elnyomhatja további bőrgyulladáshoz vezetve. Emellett a $FOXP3^+$ sejtek lehetnek átmeneti Th2-szerű effektor sejtek is, amik önmagukban is elősegíthetik az atópiás bőrgyulladást. A perifériás $FOXP3^+$ sejtek mennyisége mérhető, mely adat információt adhat a betegség súlyosságáról. (SEB: *staphylococcus* enterotoxin B; TSLP: thymus stromális lymphopoetin)

egészséges kontrollokhoz képest, és szignifikáns korrelációt mutatott a betegség súlyossági paramétereivel (25).

Hasonlóan az előzőekhez az atópiás gyulladásos bőrben talált nTreg sejtvizsgálatok eredményei is igen ellentmondásosak. Míg az egyik vizsgálat nem talált nTreg sejteket lézionális AD bőrben (26), addig egy másik nagy mennyiségű sejtet írt le a bőrben (27). Saját vizsgálataink szerint a $CD4^+CD25^+FOXP3^+$ nTreg sejtek szignifikánsan emelkedett számát találtuk atópiás bőrben, továbbá az atópiás ráhelyezési teszt (atopy patch test) pozitív AD betegek biopsziáiban olyan epidermális sejtaggregátumokat figyeltünk meg, melyek szoros kapcsolatot mutattak a $FOXP3^+$ nTreg sejtekkel, utalva ezen sejtek AD kialakulásában betöltött elengedhetetlen szerepére (28).

Az AD betegek nagy százalékában írtak le *Staphylococcus aureus* kolonizációt. Az nTreg sejtek funkcióinak változásait AD-ben vizsgálva leírták, hogy *S. aureus* jelenlétében a regulatív sejtek elvesztették az effektor T sejteken való szuppresszív hatásukat (21). Korábbi vizsgálatainkban funkcionális tesztekkel azt is bizonyítottuk, hogy az nTreg szuppresszor funkciós aktivitás homeosztatikus körülmények között az atópiás betegekben megtartott, de dózisfüggő *staphylococcus* enterotoxin B (SEB) stimuláció hatására károsodik (nTreg sejtek másodlagos funkciózavara), és a sejtek elveszítik szuppresszív képességüket mind az AD betegekben, mind az egészségesekben (25). Azaz az egyént körülvevő mikrokörnyezet (folyamatos SEB jelenlét a betegek bőrén) jelenti a legnagyobb különbséget a betegekben az egészségesekhez viszonyítva (25, 29).

Az eddig közölt tudományos eredmények alapján egy hipotézis állítható fel az nTreg sejtek lehetséges szerepéről AD-ben (*2. ábra*). Az állandóan jelenlévő allergén és bakteriális stimulusok szignifikáns effektor T sejt aktivációhoz vezetnek az AD betegek bőrében. A bőrgyulladás során a $FOXP3^+$ T sejtek helyi indukciója figyelhető meg. Ha ezek a sejtek regulatív funkcióval rendelkező $FOXP3^+$ indukált Treg-ek (25), akkor az AD-ben tapasztalt folyamatos SEB jelenlét elnyomja funkciójukat további intenzív bőrgyulladáshoz vezetve. Ha ezek a sejtek átmeneti $FOXP3^+$ Th2-szerű effektor sejtek, akkor önmagukban is képesek az atópiás bőrgyulladás elősegítésére. Ezek a $FOXP3^+$ sejtek a periférián megjelenve mérhetők, és információt adnak a betegség súlyosságáról (30, 31).

Összefoglalás

A regulatív T sejtek fontos kontroll funkciókat töltenek be az immunológiai folyamatokban, habár pontos feladatuk AD-ben még nem teljesen tisztázott. Az allergiás, autoimmun és krónikus gyulladásos kórképeket félresiklott effektor T sejt mediált immunválaszok jellemezik, melyeket részlegesen magyarázhatnak az nTreg sejtek mennyiségi és/vagy funkcionális változásai is.

A regulatív sejtek heterogenitása és a specifikus sejtmarkerek hiánya miatt azonban a különböző vizsgálatok nTreg-ek karakterizálására vonatkozó eredményei nehezen összehasonlíthatók (6, 8). Bár kezdetben feltételezték az nTreg sejtek alacsonyabb számát perifériás vérben AD során, az irodalmi adatok azt mutatják, hogy a betegek vérében az nTreg sejtek száma emelkedett, és számuk a betegség súlyosságával pozitív korrelációt mutat, mely a $FOXP3^+$ nTreg sejtek AD kialakulásában betöltött patogenetikai szerepére utal. A funkcionális tesztek bizonyítják, hogy a betegek perifériás vérében az nTreg sejtek primeren megtartják szuppresszor aktivitásukat, de SEB stimulus hatására az egészségesekhez hasonlóan elvesztik szuppresszív képességüket, azaz az egészségesekkel ellentétben, AD esetén a regulatív sejtek funkciójára a legnagyobb mértékű befolyással a beteget körülvevő mikrokörnyezet van.

A munka elvégzéséhez az OTKA K108421 pályázat nyújtott segítséget.

IRODALOM

1. *Bieber T.:* Atopic dermatitis. Ann Dermatol (2010) *22*, 125-137.
2. *Levin J., Friedlander S. F., Del Rosso J. Q.:* Atopic dermatitis and the stratum corneum: part 1: the role of filaggrin in the stratum corneum barrier and atopic skin. J Clin Aesthet Dermatol (2013) *6*, 16-22.
3. *Toda M., Leung D. Y., Molet S. et al.:* Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. J Allergy Clin Immunol (2003) *111*, 875-881.
4. *Harskamp C. T., Armstrong A. W.:* Immunology of atopic dermatitis: novel insights into mechanisms and immunomodulatory therapies. Semin Cutan Med Surg (2013) *32*, 132-139.
5. *Beck L. A., Thaci D., Hamilton J. D. et al.:* Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. NEJM (2014) *371*, 130-139.
6. *Loser K., Beissert S.:* Regulatory T cells: banned cells for decades. J Invest Dermatol (2012) *132*, 864-871.
7. *Buzás E.:* Immuntolerancia. In: Falus A, Buzás E, Rajnavölgyi É (szerk.): Az immunológia alapjai, Budapest: Semmelweis Kiadó; (2007) 169-172 p.
8. *Romagnani S.:* Regulatory T cells: which role in the pathogenesis and treatment of allergic disorders? Allergy (2006) *61*, 3-14.
9. *Akdis M., Blaser K., Akdis C. A.:* T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. J Allergy Clin Immunol (2005) *116*, 961-968.
10. *Honda T., Miyachi Y., Kabashima K.:* Regulatory T cells in cutaneous immune responses. J Dermatol Sci (2011) *63*, 75-82.
11. *Samochocki Z., Alifier M., Bodera P. et al.:* T-regulatory cells in severe atopic dermatitis: alterations related to cytokines and other lymphocyte subpopulations. Arch Dermatol Res (2012) *304*, 795-801.
12. *Caton A. J., Weissler K. A.:* Regulatory cells in health and disease. Immunol Rev (2014) *259*, 5-10.
13. *Agrawal R., Wisniewski J.A., Woodfolk J. A.:* The role of regulatory T cells in atopic dermatitis. Curr Probl Dermatol (2011) *41*, 112-124.
14. *Bangert C., Brunner P. M., Stingl G.:* Immune functions of the skin. Clin Dermatol (2011) *29*, 360-376.
15. *Wan Y. Y., Flavell R. A.:* Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. Nature (2007) *445*, 766-770.
16. *Geginat J., Paroni M., Maglie S. et al.:* Plasticity of human CD4 T cell subsets. Fron Immunol (2014) *5*, 630.
17. *Shevach E. M.:* From vanilla to 28 flavors: multiple varieties of T regulatory cells. Immunity (2006) *25*, 195-201.
18. *Wildin R. S., Smyk-Pearson S., Filipovich A.H.:* Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J Med Genet (2002) *39*, 537-545.
19. *Boguniewicz M., Leung D. Y.:* Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. Immunol Rev (2011) *242*, 233-246.
20. *Romagnani S.:* The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? Immunology (2004) *112*, 352-363.

049

Development of an *in vitro* microbiome and infection model

D van der Krieken,[1] T Ederveen,[1] P Scheepers,[2] S van Hijum,[3] J Schalkwijk[1] and P Zeeuwen[1] *1 Dermatology, Radboudumc, Nijmegen, Netherlands, 2 Health Evidence, Radboudumc, Nijmegen, Netherlands and 3 Centre for Molecular and Biomolecular Informatics, Radboudumc, Nijmegen, Netherlands*

Human reconstructed skin models are available to study defence mechanisms involved in host-microbial interactions between keratinocytes and resident commensals or opportunistic pathogens. These cellular models, however, are laborious and expensive, prone to uncontrolled bacterial overgrowth, and do not allow high-throughput screening. Therefore, we developed a completely new *in vitro* system that mimics human skin for bacterial growth, and can be used for high-throughput testing of individual microorganisms, or microbial ecology by metagenomic analysis. Microorganisms attach and live on the stratum corneum of our skin. In our newly developed model, human callus serves as substrate and nutrient source for bacteria. After 7 days of culturing, bacteria are collected for analysis. Bacterial survival is measured by colony forming units (CFU) counting and by qPCR using strain-specific primers. Prior to genomic DNA (gDNA) isolation, the bacteria are treated with propidium monoazide (PMA) and exposed to light to eliminate gDNA originating from non-viable bacteria. We succeeded to mimic in vivo conditions of human skin by infection of our model with human skin commensals (*S. epidermidis* and *P. acnes*), which survived for more than one week in vitro. Known human pathogens (*S. aureus*, *P. aeruginosa*, and *S. pyogenes*) were tested and survived in this model. Furthermore, the ratios between bacterial communities and the bacterial diversity of a human in vivo microbiome collected from the lower back of healthy volunteers remained stable after one week of culturing on the model. We envision that our experimental setup can be used as a model for skin diseases linked to microbial colonisation. Future investigations will focus on modulation of 'disease-associated microbiomes' by stimulation of 'desirable' commensal bacteria to prevent expansion of pathogenic species.

050

Mammalian target of rapamycin is increased in hidradenitis suppurativa and acne being related to insulin resistance

A Balato, S Lembo, G Caiazzo, V De Vita, R Di Caprio, M Donnarumma, G Fabbrocini and G Monfrecola *Unit of Dermatology, Department of Clinical Medicine and Surgery, University of Naples Federico II, Naples, Italy*

The aim of this study was to investigate the possible involvement of mammalian target of rapamycin (mTOR) in hidradenitis suppurativa (HS) and acne evaluating its possible relation with insulin resistance. The study population comprised 30 subjects equally distributed for HS, acne and healthy volunteers. All subjects underwent anthropometric measurements, homeostasis model assessment of insulin resistance (HOMA-IR) and oral glucose tolerance test (OGTT). RNA and protein analysis was executed on skin biopsies from non lesional and lesional skin of all HS, acne patients and on healthy skin. Our results showed that mTOR was enhanced in lesional as well as non lesional skin of HS and acne patients compared to healthy controls. Interestingly, mTOR increases correlated with insulin resistance in both analyzed diseases. Indeed, 60% of HS patients were insulin resistant and mTOR skin gene expression correlated with insulin secretion during OGTT at 30 and 60 minutes (r=0.8 and r=1, respectively). Moreover, mTOR skin gene expression significantly correlated with the severity of HS, assessed through Sartorius score (r=0.8); being the last one related to body mass index (BMI; r=1). Regarding acne patients, we found a significant correlation between HOMA-IR and mTOR skin gene expression (r=0.7). In conclusion, our findings show a possible involvement of mTOR in the complex inflammatory scenario of HS and acne as well as its potential association with insulin resistance.

051

Investigation of skin barrier functions and allergic sensitization in patients with Hyper-IgE syndrome

Z Dajnoki,[1] G Mócsai,[1] B Tóth,[2] L Maródi,[2] K Gáspár,[1] G Béke,[1] A Kapitány[1] and A Szegedi[1] *1 Division of Dermatological Allergology, Department of Dermatology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary and 2 Department of Infectious and Pediatric Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*

Hyper-IgE syndrome (HIES) is a rare, but severe primary immunodeficiency, characterized by increased serum IgE levels, recurrent infections and atopic dermatitis (AD)-like skin lesions. STAT3 mutations are detected in the most patients, which cause impaired Th17 development. AD is a chronic inflammatory skin disease with immunologic alterations and skin barrier dysfunctions. Our aim was to investigate and compare the skin barrier alterations and allergic sensitization (AS) in HIES and AD patients and to find similar or different pathogenetic events in the development of skin lesions. Analyses of STAT3 and filaggrin (FLG) mutations were performed in 6 HIES and 30 AD patients as controls. Laboratory parameters (LDH level and eosinophil count), immunologic alterations (intracellular cytokine staining), AS (total and specific IgE levels, medical history), and skin barrier changes [transepidermal water loss (TEWL), serum thymic stromal lymphopoietin (TSLP) levels] were also examined. Mutation analysis of STAT3 showed 100% positivity in HIES patients, although all of them had FLG wild-type concerning R501X and 2282del4 mutations, which were found in 31% of our AD patients in heterozygous form. No differences were found between the two diseases regarding LDH and IgE levels or eosinophil counts. Impaired Th17 cell numbers were detected in T cells of HIES patients. No altered barrier functions were found in HIES patients which were significantly impaired in AD patients. AS was more frequent in AD. On the basis of these results barrier alterations probably are not the main pathogenetic events in the development of skin lesions in HIES. Despite of the high IgE levels, AS is not a characteristic feature in these patients, which can be the consequence of their normal skin barrier functions, since outside-inside barrier impairment seems to be necessary for the development of AS.

052

Topical TRK-820 (Nalfurafine), a kappa-opioid receptor agonist, suppresses scratching and inflammatory changes in oxazolone challenged mouse ears

G Elliott,[1] M Soeberdt,[2] R Vanwersch,[1] D Metze,[3] T Lotts,[3] S Staender[3] and C Abels[2] *1 Derphartox, Delft, Netherlands, 2 Dr. August Wolff GmbH & Co. KG Arzneimittel, Bielefeld, Germany and 3 Dept. Dermatology, University Hospital Münster, Münster, Germany*

We investigated the effect of topical TRK-820 on scratching and inflammation in the oxazolone mouse ear model (inducing a chronic allergic contact dermatitis). Mouse ears were treated with 100µl of 1% oxazolone in acetone on days 0, 7, 9 and 11. On days 11 through 18 ears were treated with 50µl of betamethasone dipropionate (BMDP), TRK-820 in DMSO or DMSO alone (control). Ear thickness was measured on days 11 through 19, and scratch events (SE) and general activity (GA) were measured on days 11 and 18 for 22 hours. Mice were sacrificed on day 19 and ears processed for histological examination. Topical BMDP (0.05%) and TRK-820 (0.2% and 1.0%) accelerated recovery of ear thickness (reduced tissue oedema). Histologically, vehicle control ears were thickened and showed abundant dermal inflammation. $CD4^+$ and $CD8^+$ T-cells, neutrophils, and eosinophils had infiltrated the dermis. Mast cells were degranulated. BMDP and, to a lesser extent, TRK-820 1% significantly reduced ear thickness and cell infiltration. TRK-820 0.2% had little effect. Mouse scratching was bi-phasic, with an initial "early" SE peak within the first 2 hrs after exposure to oxazolone and a second "late" period lasting from around 18.00 hrs until early morning. Scratch frequency (Hertz) varied between 12 and 30Hz with an optimum of 16Hz. Topical BMDP (0.05%) and TRK-820 (0.2% and 1.0%) inhibited scratching compared to the vehicle control on days 11 and 18. There was also a shift in scratch frequency from higher to lower frequencies (<16Hz). There was an initial inhibition of GA for 4 hrs after the initial treatment with TRK-820 on day 11 which was not present on day 18. The strong early anti-pruritic effect of TRK-820 was transient and would appear to be related to some extent to reduced GA at day 11. Topical KOR agonists may be useful in the treatment of inflammatory skin diseases and associated pruritus.

053

IL-1 and IL-36 are the dominant cytokines in Palmar Plantar Pustulosis

A Johnston,[1] X Xing,[1] L Wolterink,[1] DH Barnes,[1] JM Kahlenberg,[2] PW Harms[3] and JE Gudjonsson[1] *1 Dermatology, University of Michigan, Ann Arbor, MI, 2 Rheumatology, University of Michigan, Ann Arbor, MI and 3 Pathology, University of Michigan, Ann Arbor, MI*

Palmar plantar pustulosis (PPP) is a chronic pustular dermatosis restricted to the palms and soles. Although rare, PPP is debilitating with a large impact on quality of life and ability to work. Resistance to treatment and disease recurrence are common thus a better understanding the pathogenesis of PPP is needed and may yield new therapeutic approaches. To delineate molecular targets in PPP and assess the pathophysiological differences between PPP and the more common chronic plaque psoriasis (CP) we analyzed archived FFPE skin biopsies of confirmed cases of PPP (n=9 control, 20 lesional) and CP (n=12 control and lesional) using Affymetrix ST 2.1 microarrays. Compared with healthy skin, PPP and CP lesions yielded 33 and CP 444 differentially expressed genes respectively (>2-fold change, FDR<.05) with 22 of these transcripts differentially expressed in both diseases. Using qRT-PCR we detected significantly elevated expression of IL1B (16x, p=.039), IL36G (7x, p=.0001), and the neutrophil chemokines CXCL1 (3x, p=.039) and CXCL2 (4x, p=.009), as well as the NADPHoxidase component NCF2 (2x, p=.04) in PPP compared with healthy palmar/plantar tissue; While IL36A, IL19 and IL8 could not be detected in healthy skin, all were significantly expressed in PPP lesions (p<.0001 all). These data were confirmed by IHC, and indicate a level of sustained activation of the IL36 and IL1 systems in PPP, which drive neutrophil infiltration. Compared with CP, PPP lesions expressed 2-fold more IL-36G (p=.01), but significantly less of the T cell chemokines CXCL9 (25x, p=.035), CXCL10 (20x, p=.038) and lower IL17A (p=.02), IL22 (37x, p=.0005) and MX1 (2x, p=.003) expression, suggesting a less prominent role for Th1/Th17 pathophysiology in PPP compared to CP. Our data may have major therapeutic implications as they suggest that the IL-1 and IL-36 inflammatory axes are the main drivers of disease pathology in PPP, and question the contribution of IL-17 and/or IFN-γ to its pathogenesis.

054

HSV-1 infection of keratinocytes inhibits inflammasome activation

H Beer,[1] G Strittmatter,[1] J Sand,[1] M Sauter,[2] M Seyffert,[3] R Steigerwald,[4] C Fraefel,[3] S Smola[2] and LE French[1] *1 Dermatology, Univerity Hospital Zürich, Zürich, Switzerland, 2 Virology, Saarland University, Homburg/Saar, Germany, 3 Virology, Univerity of Zürich, Zürich, Switzerland and 4 Infectious Disease Division, Bavarian Nordic GmbH, Martinsried, Germany*

Herpes simplex virus (HSV) is a double stranded (ds) DNA virus that is extremely well adapted to humans. HSV targets keratinocytes usually at mucocutaneous parts of the skin. In most cases infections do not cause much harm but can cause morbidity and even mortality in immunocompromised patients. Inflammasomes comprise a group of innate immune complexes, which induce an inflammatory response upon sensing of several different stress signals. This is achieved by activation of the protease caspase-1, which in turn activates the proinflammatory cytokines prointerleukin(IL)-β and -18. In human keratinocytes the NLRP3/NLRP1 inflammasome is activated by UVB irradiation whereas the AIM2 inflammasomes plays an important role in psoriasis. Transfection of human primary keratinocytes with dsDNA or infection with the dsDNA virus Modified Vaccinia Virus Ankara (MVA) induces IL-1β and -18 secretion, which is dependent on expression of proteins of the AIM2 inflammasome. In contrast, HSV-1 infected keratinocytes do not secrete IL-1β or -18. Further experiments revealed that HSV-1 infection suppresses proIL-1β expression and inflammasome activation and this effect is dependent on viral gene expression. With these results we identified inflammasome inhibition in keratinocytes as a novel immune response evade mechanism of HSV-1, which might underlie the virus' ability to cause life-long infections. However, keratinocytes can be primed to overcome inflammasome inhibition by HSV-1 infection.

216

A comparison of atrophic and hypertrophic facial photoageing

I Ayer, RE Watson, T Griffiths and CE Griffiths *The Dermatology Centre, Salford Royal NHS Foundation Trust & The University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom*

Photoageing is due to the cumulative effects of sun exposure superimposed on chronological cutaneous ageing. Clinically, two main phenotypes of facial photoageing exist: atrophic smooth telangiectactic skin (AP) and; hypertrophic wrinkled skin (HP). AP is prone to the development of non-melanoma skin cancers (NMSC). The mechanisms that underpin these two disparate phenotypes are poorly understood. The aim of this study was to evaluate the histological differences between phenotypes by examining: 1) the morphology of epidermis and dermal epidermal junction (DEJ) and; 2) the composition and distribution of 4 relevant biomarkers [elastic fibres; fibrillin-rich microfibrils (FRM); collagen VII and; von Willebrand Factor (vWF)] in the dermal extracellular matrix from subjects with either AP or HP [n=20 per group (mean ± SE); AP (78.7y ±2.02); HP (74.5.6y ±2.08)]. We found that AP epidermis was thicker than HP ($P< 0.0001$) but there were no significant differences in DEJ convolution between phenotypes ($P>0.05$). The percentage of dermis occupied by mature elastin fibres was significantly greater in HP than AP ($P<0.0001$), but the dermis of HP was less enriched in FRM than AP ($P<0.05$). AP was found to be collagen VII-poor compared to HP ($P<0.05$) but, as expected, was more vascular with a greater number of blood vessels ($P<0.001$ & $P< 0.0001$, respectively). AP had significantly more NMSC (n=25) than HP (n= 0). No differences were found in any of these biomarkers in sun-protected buttock skin obtained from the same patients. This study demonstrates that the stroma in AP facial skin is characterised by less solar elastosis and collagen VII expression and more FRM, increased vascularisation and NMSC as compared to the HP phenotype. HP and AP appear to be distinct clinical and histological entities. Further investigation is warranted to determine the underlying mechanisms in HP that confer protection against development of NMSC.

217

The wet-wrap method with diluted corticosteroids versus emollients in children with atopic dermatitis – a prospective, randomized, double-blind, placebo controlled trial

SR Janmohamed,[1] J Gutermuth[1] and AP Oranje[2] *1 Dermatology, University Hospital Brussels (UZ Brussel), Brussels, Belgium and 2 KinderHaven, Rotterdam, Netherlands*

Atopic dermatitis (AD) can be treated in multiple ways with both local and systemic therapy. In children with severe AD wet-wrap therapy (WWT) is a safe option compared to systemic therapy with many side effects. WWT is performed in various ways, with or without (diluted) corticosteroids. The question remains whether diluted corticosteroids are really necessary, however, a comparative study has never been done before. We performed a prospective, multicentre, placebo controlled, double-blind, randomized clinical trial. Patients between 6 months and 10 years old with severe AD (SCORAD > 40) who were eligible for WWT were asked to participate in this study. Treatment consisted of 4 weeks of WWT with either verum (diluted corticosteroid: mometasone fuorate 1:3 body and 1:19 face) or placebo (petroleum 20% in cremor cetomacrogolis). Both groups were treated in the same way: week 1 - apply once daily on the whole body using the fingertip unit method; week 2-4 - apply once daily on the lesions at 4 consecutive days per week, using the fingertip unit method. At d0, d1, d4, d7, d14, and d28 outcome measures (SCORAD, Quality of life scores, and adverse events) were gathered. A total of 39 patients were included in the study; after randomization 20 in the placebo group and 19 in the verum group. The SCORAD decreased in both groups, but better and faster in the verum group (mean difference in SCORAD at all time points = 13.2, $p<0.0001$). Side effects were mild in the verum group: 10x folliculitis and 3x decrease of cortisol level in the first week, which returned to normal after a re-check in the second week. In the placebo group we observed 2x folliculitis and 2x infected AD. Both groups show an improvement of AD, however, the diluted corticosteroids group shows a statistically significant faster improvement, especially in the first week of treatment.

218

Allergen specific immunotherapy in atopic dermatitis

A Kapitány,[2] S Baráth,[1] A Khasawneh,[2] G Béke,[2] Z Dajnoki,[2] Z Káplár,[2] A Szegedi[2] and K Gáspár[2] *1 Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary and 2 Division of Dermatological Allergology, Department of Dermatology, Faculty of Medicine, University of Debrecen, Derbrecen, Hungary*

Atopic dermatitis (AD) is a chronic inflammatory skin disease prone to relapse, having both genetic and environmental factors (e.g. allergens) as underlying causes for its development. The use of allergen specific immunotherapy (ASIT) has been restricted to insect venom allergy, allergic rhinitis and mild extrinsic asthma; however a few contradicting results are available on the use of ASIT in AD. Our aim was to analyze the effect of ASIT on clinical and subclinical variables in patients sensitized by house dust mite and suffering from both allergic rhinitis and AD. We examined the patients clinical (physical status, disease specific questionnaire), immunological laboratory (defining regulatory and effector T cells and also blood dendritic cells – flow cytometry; determining serum allergen specific IgE levels – ELISA; atopy patch test, prick test) as well as skin barrier (specifying Filaggrin mutation – molecular genetics; determining serum TSLP levels – ELISA; measuring TEWL – Tewameter) parameters prior to and during the ASIT treatment in comparison with diseased control groups. As a result of ASIT the measured clinical and skin barrier variables displayed improvement compared to the initial values as well as to the control group, although the differences were not significant. However when only patients without filaggrin mutation where compared, the ASIT treated patients showed significant improvement in barrier (TEWL) function (38,96+17,42 g/m^2h vs. 19,97+2,077 g/m^2h; p=0,0357). This result predispose that the modified immune status may improve the skin barrier in the mild-to-moderate patients without filaggrin mutation. Perhaps this AD population may benefit of ASIT. There is an intricate pathogenesis underlying AD, which requires a complex approach in therapy. Additionally to the previously described immunological changes attributed to ASIT, analysis of other physicochemical barrier parameters is crucial.

219

Interventions for nail psoriasis

C Busard,[1] M Pasch,[2] S Oudshoorn,[1] L Hooft[1] and PI Spuls[1] *1 Academic Medical Center, Amsterdam, Netherlands, 2 Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands and 3 Dutch Cochrane Centre, Utrecht, Netherlands*

In this update of a Cochrane Review we systematically assess the efficacy and safety of nail psoriasis treatments. The search was updated to November 2014 in Cochrane Skin Group Specialised Register, CENTRAL, MEDLINE, Embase, LILACS and ongoing trial registers. Twelve new studies (1469 patients) were included making a total of 30 studies comprising 2735 patients. Five biologic, 3 radiotherapy and 3 topical studies reported statistically significant improvements in nail disease severity. Short term improvement was demonstrated for ustekinumab 45 and 90mg vs placebo (resp. 26.7% and 24.9% vs 11.8%), secukinumab 150mg monthly and early dosing vs placebo (resp. 10.6% and 19.5% vs -14.4%), superficial radiotherapy vs sham radiotherapy (20% vs 0% nail), lindoil vs olive oil (59.3% vs 16.3%) and tacrolimus 0.1% vs no active treatment (56.2% vs 15.5%). Medium term improvement was demonstrated for infliximab 5 mg/kg (57.2% vs -4.1%), golimumab 50 and 100 mg (resp. 33% and 54% vs 0%), certolizumab (58.8% vs 42.4%) and HPCH lacquer vs placebo (55% vs 31.7%). For ustekinumab and golimumab long term extension studies indicate maintained clinical response during 52 and 256 weeks respectively. A higher incidence of adverse events (mild and tolerable) was reported for systemic compared to topical and radiotherapy. In addition to infliximab and ustekinumab newly introduced biological agents (secukinumab, golimumab and certolizumab) have shown to be beneficial in the treatment of nail psoriasis. Radiotherapy for psoriasis is not used in common practice. Although the quality of evidence for topical therapy was generally poor, most interventions showed some improvements on nail disease severity and could be valuable in clinical practice if systemic therapy is not indicated. With the growing amount of upcoming clinical trials and the development of new scoring systems, harmonization of outcome measures and clinical homogeneity is essential to enhance comparability between clinical trials and to improve the body of evidence for nail psoriasis treatments.

220

Stress markers in chronic urticaria

A Ograczyk,[1] M Kozlowska,[2] A Kaszuba[2] and A Zalewska-Janowska[1] *1 Psychodermatology Department, Medical University of Lodz, Lodz, Poland and 2 Dermatology, Pediatric Dermatology and Oncological Dermatology Clinic, Medical University of Lodz, Lodz, Poland*

It is well known that environmental stressors, including psychological ones, play an important role in triggering chronic urticaria and could influence its course. Our study aim was to compare selected biological parameters (cortisol and DHEA-S saliva levels and cortisol/DHEA-S ratio - concerned as stress markers) with psychological ones (stress level and quality of life) between chronic urticaria patients and controls. The study group recruited at dermatology departments, consisted of 46 females suffering from chronic urticaria (age range: 21-68 years, mean±SD 44.6±14.2) and 33 females concerned as our control group (age range: 18-78 years, mean±SD 46.3±14.7). Disease duration ranged from 6 weeks to 25 years (mean±SD 3.6 years±5.8 years). The following methods were used: Perceived Stress Scale (PSS-10), The Short Form Health Survey (SF-36), and ELISA method to assess saliva cortisol and DHEA-S levels. The statistical significance was set at $p<0.05$. Patients with chronic urticaria presented lower cortisol ($z=6.833$, $p<0.001$) and DHEA-S levels ($z=7.024$, $p<0.001$) in comparison to the control group.The obtained results could be regarded as objective confirmation of negative influence of chronic stress in urticaria patients (PSS-10 results are compatible with them). Patients with chronic urticaria revealed also worse quality of life (QoL) in reference to all SF-36 dimensions, except for bodily pain. The results revealed statistically significant correlation between higher DHEA-S level ($r=0.39$, $p<0.01$), lower cortisol/DHEA-S ratio ($r=-0.36$, $p<0.05$) and better physical functioning in patients with chronic urticaria. In this group stress also negatively correlated with QoL – with all SF-36 dimensions. Our results confirmed negative role of stress in chronic urticaria course which led to worse physical, mental and social functioning. Chronic influence of stress was revealed also in biological parameters, which –by definition– are regarded as fairly objective methods.

221

Scoring infantile haemangiomas: the Haemangioma Activity Score

SR Janmohamed,[1] L De Raeve,[1] J Gutermuth[1] and AP Oranje[2] *1 University Hospital Brussels (UZ Brussel), Brussels, Belgium and 2 Erasmus MC, Rotterdam, Netherlands*

Until recently, scoring systems for infantile haemangioma (IH) activity were lacking. We developed the Haemangioma Activity Score (HAS), a simple scoring system for retrospective and prospective use, based on colour, swelling, and ulceration. Recently, the Haemangioma Severity Scale (HSS) has also been described. The following studies were undertaken to validate the HAS and to compare the HAS with the HSS. We validated the HAS with a retrospective observational study of photographs of IHs. We selected those patients who had clear and representative photographs and had a follow-up of at least six months. To assess agreement, the HAS of these n=78 IHs was calculated independently at two time points by three physicians. We calculated the intraclass correlation coefficients (ICC) of the HAS at t=0 and at t=1. In a prospective interventional study with 54 infants with IHs treated with oral propranolol we compared the HAS with the HSS. The HAS and the HSS were applied independently by two observers. Mean ICC's of three observers of the HAS at t=0 and t=1 were 0.72 and 0.76, respectively. We noted that HSS scores often remained the same upon improvement of the IH and therefore do not reflect severity. HAS scores decreased over time, with a dramatic drop in the first week of treatment, reflecting the immediate therapeutic responses. We conclude that the HAS is a promising scoring system for scoring the activity of IHs in patients at different time intervals. It could be useful in future investigations for examining the activity of IHs after various therapies or for following the natural course. We also conclude that the HAS is to be preferred over the HSS. Advantages: the HAS can also be used in patients with deep IHs and can be used both prospectively with patients and retrospectively on photographs.

455

The role of orphan receptor Interleukin-17 Receptor D in the pathogenesis of psoriasis.

M Mellett,[1] F Truzzi,[2] PN Moynagh,[3] C Pincelli,[2] E Contassot[1] and LE French[1] *1 Department of Dermatology, University Hospital Zurich, Zurich, Switzerland, 2 University of Modena and Reggio Emilia, Modena, Italy and 3 Biology Department, Maynooth University, Maynooth, Ireland*

Interleukin 17 Receptor D (IL-17RD), also known as Similar expression to FGF genes (SEF), is an orphan receptor of the IL-17 receptor family, having no known ligand. We previously reported that IL-17RD differentially regulates IL-17A signalling pathways by directly targeting IL-17 receptor adaptor protein, Act1, by SEF / IL-17R (SEFIR)/SEFIR interactions, which leads to downregulation of NF-κB and ERK activation but increased p38 MAPK activity. This makes IL-17RD a potential target for developing therapeutics for disease where IL-17 signalling is implicated, including psoriasis. Indeed, two separate single nucleotide polymorphisms in the Il17rd gene have recently been linked to an augmented risk of Crohn's disease and increased psoriasis susceptibility. Il17rd gene expression has also been shown to be down-regulated in psoriatic lesions. Therefore, it is of interest to characterise a role for IL-17RD in psoriasis pathogenesis. Here we demonstrate that the regulatory effects of IL-17RD are not restricted to IL-17 signalling but IL-17RD also targets Toll-like receptor-induced signalling pathways. In primary human peripheral blood mononuclear cells (PBMCs), knockdown of Il17rd mRNA levels leads to enhanced pro-inflammatory signalling and gene expression. IL-17RD-deficient mice were also more susceptible to TLR-induced septic shock and pro-inflammatory cytokine-induced inflammation. We assessed IL-17RD protein expression in psoriatic tissue by immunohistochemistry staining and observed decreased IL-17RD protein expression in the epidermis of psoriasis patients compared to controls. Interestingly, this loss of Il17rd expression also correlated with higher expression of pro-inflammatory molecules. Taken together our data suggest that IL-17RD is an important regulator of the pro-inflammatory pathways that contribute to psoriasis pathogenesis.

456

Unraveling leukocyte populations in adipose tissue

R Naito,[1] M Brüggen,[1] W Bauer,[1] M Zeyda,[3] F Koszik,[1] H Kiprov[2] and G Stingl[1] *1 Dept. Dermatol., DIAID, Medical University of Vienna, Vienna, Austria, 2 Private Clinic Kiprov, Vienna, Austria and 3 Dept. Internal Medicine III, Medical University of Vienna, Vienna, Austria*

Adipose tissue (AT) had long been regarded as a site of inert lipid/energy storage. More recent evidence exists that AT can be both source and target of various inflammatory cytokines and adipokines. In order to determine the potential cellular source(s) of such mediators, we sought to immunophenotypically characterize the leukocytic infiltrate of AT. Therefore, we collected AT from abdominoplasty surgeries as well as from liposuctions. Multicolor immunofluorescence stainings of tissue sections and flow cytometry of isolated cells were used to characterize residing immune cell populations in AT. For FACS, cells were obtained from AT specimens using a collagenase I digestion followed by a Ficoll gradient. Our FACS analyses revealed that 70% of the isolated cells were stromal cells, i.e. fibroblasts, endothelial cells and preadipocytes. The remaining 30% were leukocytes (defined by the expression of CD45). CD3 positive T lymphocytes constituted about 20% of the overall leukocyte population. On AT tissue sections, these CD3+ T cells were mostly located in the interlobular AT septa. The vast majority of T cells expressed the memory T cell marker CD45RO, whereas only 2% showed a naive T cell phenotype (CD45RA). Interestingly, 20-30% of CD3+CD45RO+ T cells expressed the skin homing marker, CLA. In contrast to T cells, B cells (CD19+) were virtually absent. 60% of CD45+ cells expressed CD14. The majority of these macrophages expressed the 'M2' marker CD206 . Mast cells, basophils, NK cells as well as other innate lymphoid cell populations were also present. In conclusion, our data revealed a prominent leukocytic infiltrate in AT and constitute the basis for next exploring the inflammatory processes in AT of chronic inflammatory diseases such as obesity and psoriasis.

457

Ex vivo and in vivo studies support safety and non-invasiveness of hair follicle targeting for transcutaneous immunization strategies

A Vogt,[1] S Hadam,[1] E Pfannes,[1] B Combadiere[2] and U Blume-Peytavi[1] *1 Department of Dermatology, Charite-Universitaetsmedizin Berlin, Berlin, Germany and 2 Centre d'Immunologie et des Maladies Infectieuses, Université Pierre et Marie Curie, INSERM U543, Paris, France*

Non-invasive targeting of cutaneous antigen-presenting cells does not only offer practical advantages. Our previous clinical trials with transcutaneously (t.c.) administered influenza vaccine using a skin surface method which targets hair follicles (CSSS) indicate, that while rather poor in induction of humoral responses, t.c. immunization may be helpful for the induction of cytotoxic T cell responses. In ex vivo studies, CSSS removed only 30% of stratum corneum material, but activated Langerhans cells and significantly increased the penetration of 200nm polystyrene particles deep into hair follicles. In this in vivo study in 12 volunteers, we assessed skin barrier function and integrity after CSSS compared to conventional adhesive tape stripping. We found no indication for marked inflammation within the first 48 hrs after the procedure assessed by clinical examination and presence of inflammatory cytokines (IL1aplha, IL-6 or IL-8) in skin surface material. TEWL values were markedly increased shortly after CSSS and adhesive tape which dropped quickly after the procedures. Return to baseline was slightly delayed in CSSS-treated areas. In accordance to the larger amount of stratum corneum removed by CSSS we observed stronger decreased pH values in CSSS treated skin areas slowly recovering over time compared to tape-stripped skin. No changes in skin elasticity was observed. Volunteers indicated in the questionnaires that they would readily choose CSSS-based TC vaccination as vaccination method if available. These observations make CSSS a highly interesting technology for patch immunizations, because the size of viruses, virus-like-particles, and nanocarriers favours hair follicle penetration, because immune activation enhances uptake activity, and because we previously found hair follicles to be key sites for cellular uptake of carriers and candidate vaccines.

458

IFNg/IL-17 cytokine milieu is characteristic of papulopustular rosacea

G Béke,[1] Z Dajnoki,[1] G Mócsai,[1] A Kapitány,[1] K Gáspár,[1] K Hajdu,[1] D Törőcsik,[2] I Kovács,[3] T Bíró[4] and A Szegedi[1] *1 Division of Dermatological Allergology, Department of Dermatology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, 2 Department of Dermatology, Debrecen, Hungary, 3 Department of Pathology, Debrecen, Hungary and 4 Departments of Immunology and Physiology, Debrecen, Hungary*

Rosacea is a common chronic inflammatory skin condition typically occurring on sebaceous gland rich (SGR) skin areas; its pathophysiology is quite complex and poorly understood. We sought to investigate and compare the innate and adaptive immune cell components and cytokine profile of papulopustular rosacea (PPR) and SGR skin. 10 PPR and 10 healthy SGR skin biopsies were gained which were used for immunohistochemistry to detect $CD3^+$ / $CD4^+$ T cells, $CD11c^+$ dermal myeloid DCs, $CD1a^+$ Langerhans cells, $CD163^+$ / Factor $XIII^+$ macrophages. Immunostaining of cytokines mainly but not exclusively characteristic of T cells, namely IL-10, IL-13, IL-17 and IFNg was also carried out. May-Grünwald-Giemsa (MGG) routine staining was also performed to compare the number of eosinophils, neutrophils and mast cells. Cell counts were quantified by Pannoramic Viewer software. Biopsies were also used to measure IL-10, IL-13, IL-17 and IFNg gene expression by qPCR. In PPR samples infiltrating DCs, T cells and macrophages were detected in significantly higher numbers compared to SGR skin. The number of Langerhans cells showed no difference. MGG staining revealed that several neutrophils were present in PPR samples, but similar to SGR skin eosinophils were missing. Cell count of mast cells was elevated compared to SGR skin. Cytokine milieu of PPR skin represented significantly elevated number of $IL\text{-}10^+$ and $IL\text{-}17^+$ cells compared to SGR skin samples and strong IFNg positivity also turned up. The gene expression pattern of the cytokines corresponded to our results on protein levels. In conclusion inflammatory IFNg/IL-17 cytokine milieu could be a crucial factor in the pathogenesis of PPR.

459

An insight into the expression patterns of antimicrobial peptides psoriasin, RNase7, HBD-2,-3 and LL37 in polymorphic light eruption.

V Patra,[1] G Mayer,[1] U Schmidbauer,[2] A Gruber-Wackernagel,[1] M Horn[2] and P Wolf[1] *1 Research Unit for Photodermatology, Medical University of Graz, Graz, Austria and 2 Department of Dermatology, Medical University of Graz, Graz, Austria*

Polymorphic light eruption (PLE) is a common photodermatosis that has been linked to immunological abnormalities. Previous work of normal skin of PLE patients has indicated that there is an abnormal regulation of antimicrobial peptides (AMPs) to UV exposure that may be involved in the pathogenesis of the disease (Felton S et al, Photochem Photobiol Sci 2013; 12(1):29-36). AMPs are best known in fighting against a wide range of gram-negative and gram-positive bacteria, as well as fungi and few viruses. Recent studies indicate that AMPs not only act against microorganisms, but also are actively involved in adaptive immune responses and can modulate immune reactions. Our study focuses on AMPs in lesional skin of PLE. We performed immunohistochemistry for psoriasin, RNase7, HBD-2, -3 and LL-37 on tissue sections of archived, paraffin-embedded skin samples from PLE lesions (n=5-12) and compared the results to that from samples (n=5-13) of sun-exposed or not-exposed adjacent healthy skin available from surgical excision of various skin tumours. Intensity of staining and/or number of positive cells were quantified by semi-quantitative scores. We observed significant expression of psoriasin, RNase7, HBD-2 and reduced expression of HBD-3 in PLE lesions compared to the healthy skin. Interestingly, LL-37 was highly expressed around blood vessels and sweat glands in PLE lesions but not in healthy skin. The expression patterns observed could be due to UV exposure, which is known to upregulate certain AMPs. Since these AMPs are known to have immunomodulating properties, our observations of differentially expressed AMPs suggest that they may indeed play a role in the pathophysiology of PLE.

460

Mast cells are increased in intrinsically aged skin and are inversely related to Langerhans cell numbers

SM Pilkington,[1] S Abdu,[1] RE Watson,[2] RJ Dearman,[1] I Kimber[1] and CE Griffiths[2] *1 Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom and 2 Faculty of Medical and Human Sciences, University of Manchester, Manchester, United Kingdom*

Aged skin exhibits a functional decline in immunity, associated with increased susceptibility to infection and malignancy, alongside an increased inflammatory status. A reduced number of antigen presenting cells, Langerhans cells (LC), is thought to contribute to these effects, but little is known about changes in other cutaneous immune cell populations during ageing. This study aimed to assess differences in leukocyte populations between young and aged skin. Skin biopsies from photoprotected buttock skin of six young (median age 26yrs; range 23-29yrs) and five aged (75yrs; 75-77 years) White Caucasians were taken and immunostaining performed on skin sections to identify neutrophils (elastase), macrophage (CD68), mast cells (tryptase), LC (CD1a), $CD4^+$ and $CD8^+$ T cells. Cells were enumerated by light microscopy and were mainly localised to the dermis, except for LC which were present in dermis and epidermis. No differences in macrophage and $CD4^+$ T cell numbers were observed between young and aged skin, whereas $CD8^+$ T cell numbers appeared higher in aged compared with young skin (mean (± SEM) of 22.6 ± 6.3 cells/high power field (hpf) vs 13.4 ± 2.9; not significant). Mast cell numbers were higher in aged compared with young skin (50 ± 4.8 cells/ hpf vs 25 ± 2.8 cells/hpf; $p<0.01$): total LC numbers were lower in aged compared with young skin (18 ± 1.0 cells/hpf vs 31 ± 5.8 cells/hpf; $p<0.05$). Moreover, mast cell numbers were found to correlate inversely with LC numbers ($r=-0.86$; $p<0.05$), while no correlations were observed between LC and the other skin resident immune populations. Intrinsically aged skin exhibits alterations in leukocyte populations which may contribute to declining immune function. A reduction in LC number may be associated with diminished regulatory control of immune responses and could contribute to elevated numbers of cutaneous inflammatory cells such as mast cells. Further studies in larger subject groups are warranted.