product shows opsonic capacity and ADCC activity in amounts comparable to other marketed IVIGs.

CONCLUSIONS: This newly developed IVIG is a highly purified, unmodified IgG therapeutic concentrate. IgA content, as well as of other contaminants, is practically negligible. The IgG concentrate has full Fc integrity and demonstrated in vivo neutralizing capacity against several human pathogens.

Funding: Instituto Grifols, S.A

1028 Human Keratinocytes Secrete TNFa Stimulated With Cytomix and *D. Pteronyssinus* Extract

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RATIONALE: The epidermal skin barrier can be damaged by proteases from *D. pteronyssinus* (Dp) and *Staphylococcus aureus*. TNFα expression and secretion by inflammatory cells occurs in atopic dermatitis (AD) and keratinocytes have been shown to secrete TNFα in the presence of staphylococcal superantigen. Since Dp has been shown to be associated with AD, this study was designed to evaluate if Dp and staphylococcal superantigen, enterotoxin B, may stimulate keratinocyte TNFα secretion.

METHODS: Confluent monolayers of human keratinocytes (HK, ATCC CRL 2309) were stimulated with a cytomix composed of 100 μg/ml staphylococcal enterotoxin B and 10U/μl rhIL-1β. Additional experiments stimulated HK with standardized dialyzed extract of 1000 AU/ml Dp. HK were stimulated with cytomix ± Dp for 24 hours in serum-free media (SFM) using standard cell culture conditions and media was assayed for TNFα. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] cell viability and proliferation assay was conducted. Parametric data were analyzed by the two-tailed t test ($\alpha = 0.05$) relative to control (SFM without cytomix and Dp).

RESULTS: HK showed >95% viability by the MTT incorporation test. HK did not increase TNFα secretion in the presence of cytomix alone, or Dp alone, relative to the control ($p>0.05$). In contrast, cytomix plus Dp stimulated a 2- to 12-fold increase in TNFα secretion by HK relative to control ($p = 0.0001$).

CONCLUSIONS: Cytomix with the addition of Dp stimulated HK secretion of TNFα over a wide range. Cytomix or Dp alone had no effect on HK secretion of TNFα. These data suggest Dp may exacerbate the symptoms of AD caused by inflammatory cytokines and staphylococcal enterotoxin B superantigen found in AD.

Funding: Novartis Pharmaceuticals

1029 Capacity of Human Intravenous Immunoglobulin (IGIV3I) Production Process to Eliminate an Experimental TSE-Model Agent

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RATIONALE: Variant Creutzfeldt-Jakob disease (vCJD) is a transmissible spongiform encephalopathy (TSE) mainly present in United Kingdom (UK). The agent (PrP^{Sc}) is a prion mainly located in the Central Nervous System, however it can also be found in the lymphatic system and non-neural tissues at much lower concentration. The vCJD agent has never been detected in blood with methods capable of detecting it in other tissues, so if present, the concentration must be very low. There are 3 possible transmissions of the vCJD agent by transfusion of cellular blood components, but such transmissions have never been reported for plasma-derived products, possibly because of the negligible prevalence of the disease outside of UK and the demonstrated prion elimination capacity of the production processes. Grifols has studied the capacity of IGIV3I production process to remove an experimental TSE model agent.

METHODS: PEG (polyethylene glycol) precipitation and nanofiltration were studied on a laboratory model. Hamster Scrapie strain 263K was spiked into intermediate process materials to determine PrP^{Sc} removal through the purification process using western blot.

RESULTS: PEG precipitation removed from $\geq 4.08\ log_{10}$/ml to $\geq 5.14\ log_{10}$/ml PrP^{Sc} and nanofiltration removed $\geq 3.3\ log_{10}$/ml. No residual agent was detected after any of these two independently studied steps. The overall clearance of the entire process would be $\geq 18.4\ log_{10}$/ml.

CONCLUSIONS: The great removal capacity of the production process indicates that low levels of vCJD agent, if present in plasma, would be eliminated through the purification process of IGIV3I.

Funding: Instituto Grifols, S.A.

1030 Sophorolipids Decrease IgE Production in U266 Cells by Downregulation of BSAP (Pax5), TLR-2, STAT3 and IL-6

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RATIONALE: Sophorolipids are promising modulators of the immune response. We have previously demonstrated that sophorolipids (1) decreased sepsis related mortality at 36 hr in vivo in a rat model of septic peritonitis by modulation of nitric oxide, adhesion molecules and cytokine production and (2) decreased IgE production in vitro in U266 cells possibly through affecting plasma cell activity. In this study we investigated the mechanism(s) involved in sophorolipid mediated downregulation of IgE in vitro in a cellular model of allergic disease.

METHODS: Log phase U266 (IgE producing myeloma) cells were cultured in complete RPMI medium (cRPMI) ± increasing concentrations of sophorolipids (0.1-100 μg/ml) for 24-72 hours after which levels of intracellular and extracellular IgE and IgA (for class switching) were determined in culture supernatants (flow cytometry, ELISA), cells were assessed for proliferation (MTT), cellular apoptosis (Annexin 5, Caspase 3) and changes in cell structure (confocal microscopy) and morphology (plasma cells/field), and compared with controls (cRPMI, 20% sucrose vehicle). mRNA expression was determined for FcεRI, IL-6, IL-6R, STAT3, TLR-2, BSAP (PAX5) with β-actin as a control (RT-PCR). Cell viability was determined by Trypan blue exclusion dye (>95% at all times). Data are reported as mean±SD and significance between groups was determined by student's t-test.

RESULTS: U266 cells cultured in cRPMI or sucrose vehicle produced high levels of IgE (508 IU±4). Addition of sophorolipids decreased intracellular and extracellular IgE production at 24-72h in a dose dependent manner (63% reduction at 100ug/ml; $p<0.001$) and correlated with an increase in the percentages of plasma-like cells compared with controls (5-fold; $p<0.05$). Furthermore, sophorolipid treatment decreased mRNA expression of BSAP(Pax5) at 24-72h, TLR-2 at 24-48h and STAT3 and IL-6 at 24h, when compared with untreated/vehicle controls; β actin was not affected. Sophorolipid treatment did not affect cellular structure, proliferation, apoptosis, IgA production, FcεRI, or IL-6R mRNA expression when compared with controls.

CONCLUSIONS: Sophorolipids decrease IgE production in U266 cells by downregulating important genes involved in IgE pathobiology in a synergistic manner. These data continue to support the utility of sophorolipids as an anti-inflammatory agent and novel potential therapy in diseases of altered IgE regulation.

Funding: NY State research funds