EFFICACY OF A NOVEL ALPHA-INTERFERON-ASSOCIATED MOLECULE (HYBRID 1) FOR THE TREATMENT OF PSORIASIS/ATOPIC DERMATITIS

Atopic dermatitis (AD) and Psoriasis are inflammatory skin disorders that impose considerable burdens upon patients' quality of life, health outcomes, and work productivity. Atopic dermatitis affects 11%-30% of children and 2%-10% of adults, depending on the population. Atopic dermatitis involves erythematous patches that are not well demarcated and are often excoriated and associated with severe pruritus. The prevalence of psoriasis is approximately 1%-3% among both children and adults, although it is uncommon before age 9. Psoriasis presents with well-demarcated plaques covered with silvery white scales with variable levels of pruritus and changes in fingernails and toenails. Compared with AD, more targeted systemic therapeutics are widely available for Psoriasis—perhaps because of an earlier understanding of its pathogenic pathways. Despite important clinical and mechanistic distinctions, the two disorders share common mechanisms of disease, including infiltration of circulating immune cells in the skin, altered expression of pro-inflammatory cytokines, and variable barrier alterations. Atopic dermatitis and psoriasis are becoming increasingly recognized as systemic rather than localized cutaneous diseases because skin inflammation is often complicated by metabolic, cardiovascular and other comorbidities. Further, AD and psoriasis are associated with a major financial burden on the healthcare system, and these associations intensify the need for novel interventions.

A number of new biological molecules, largely cytokine-specific monoclonal antibodies, have been introduced over the past 3 years that appear to have revolutionized the systemic treatment of Psoriasis. Unfortunately, however, this is not always the case with Psoriasis as often the therapy becomes less effective with time and AD where we still await therapies that simultaneously target the multiple pathways involved in its pathogenesis.

FIGURE 1. **Psoriasis Therapy Discontinuation**.

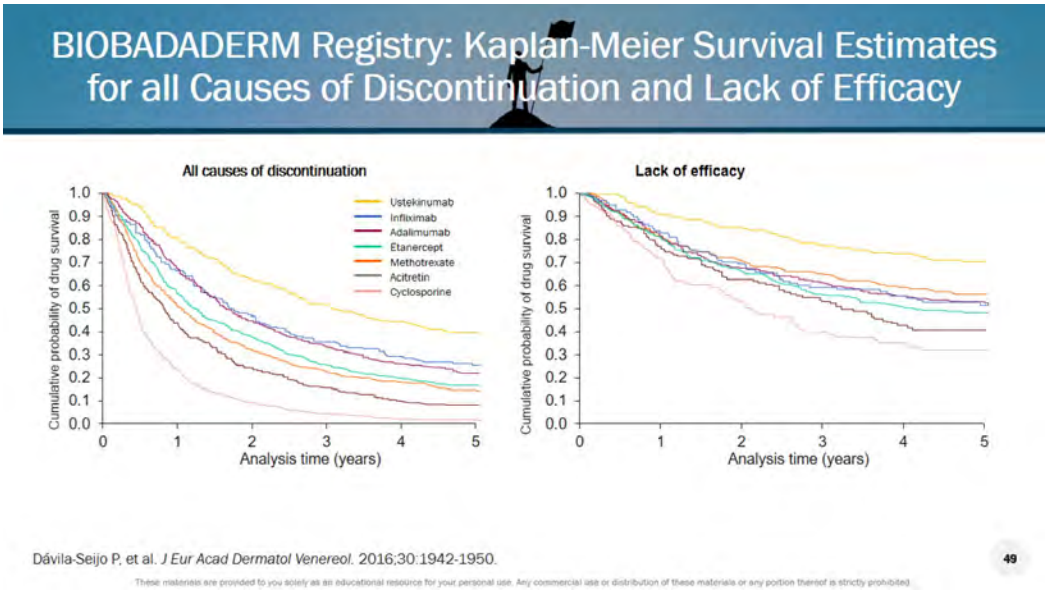


FIGURE 2

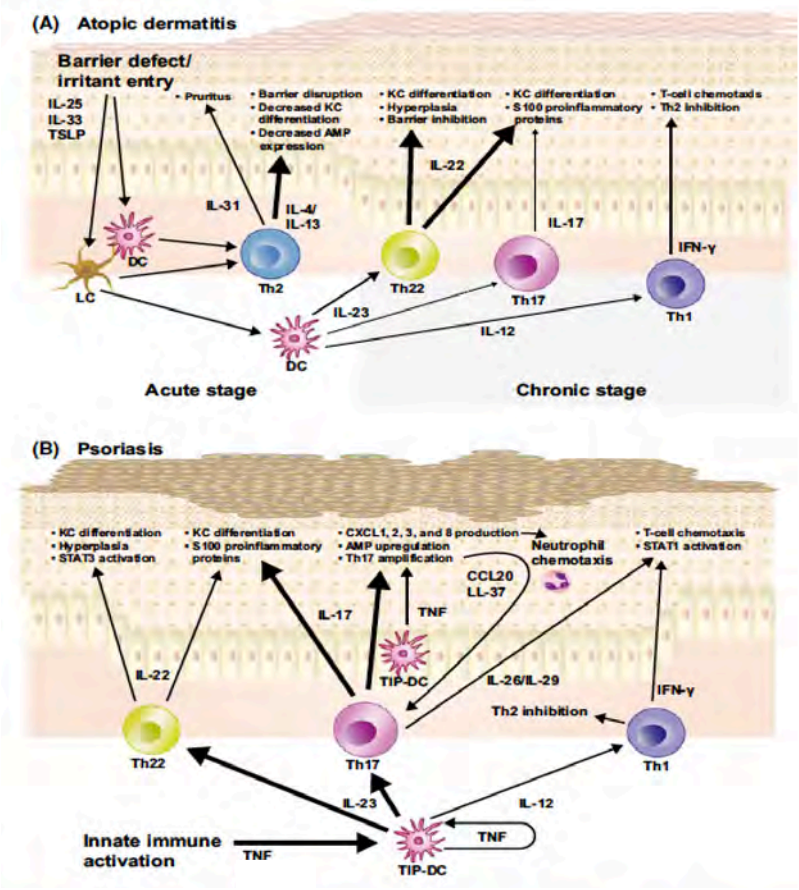


FIGURE 2 **Immune mechanisms of atopic dermatitis and psoriasis**.
(A) **Atopic dermatitis** lesions are characterized by dendritic cells and Langerhans cells activated from keratinocytes. Activated dendritic cells stimulate
Th2 cells to produce IL-4, IL-13 and IL-31, which induce changes in keratinocyte gene expression, disruptions in barrier function and itch symptoms. Transition from the acute (days 1-3) to the chronic state (day 3 onward) is marked by recruitment of
Th22 and Th17 subsets, which induce keratinocyte responses that attract additional circulating immune cells to the epidermis, alter keratinocyte differentiation and induce epidermal thickening.
(B**) Psoriasis** pathology begins with the activation of IL-23- releasing dendritic cells, characteristically by TNF-associated systemic inflammation. Activation of Th17 cells by IL-23 leads to IL-17- mediated keratinocyte responses that attract circulating neutrophils, increase AMP expression and produce factors that promote self-amplifying feedback to the Th17 population. Activation of Th22 cells by IL-23
induces the expression of keratinocyte genes that influence differentiation and the initiation of hyperplasia. Interleukin-22 also works in synergy with IL-17 to alter keratinocyte differentiation and increase S100 protein expression. AMP, antimicrobial protein; CCL, chemokine (C-C motif) ligand; CXCL1, 5, 8, chemokine (C-X-C motif) ligand; DC, dendritic cell; IFN, interferon; IL, interleukin; KC, keratinocyte; LC, Langerhans cell; LL-37, cathelicidin peptide; STAT, signal transducer and activator of transcription; Th, T helper; TIP-DC, tumour necrosis factor and inducible nitric oxide synthase producing dendritic cell; TNF, tumour necrosis factor; TSLP, thymic stromal lymphopoietin.
[from: Guttman-Yassky E, Krueger JG,Lebwohl MG. Systemic immune mechanisms in atopic dermatitis and psoriasis with implications for treatment. Exp Dermatol.2018;27:409–417. https://doi.org/10.1111/exd.13336

Atopic dermatitis (AD), also called eczema, is a chronically relapsing skin disease. It is prevalent in approximately 10.7% and 7.2% of US children and adults, respectively. The onset of AD is usually in early childhood, and it can have an impact on the entire family unit. Additionally, AD is increasingly recognized as a disease that often persists into or begins in adulthood. Thus, AD can have a detrimental effect on the lives of patients and their families throughout the lifespan. This includes impacts on quality of life and social, academic, and occupational impacts. AD places a tremendous financial burden on patients, their families, and society as a whole through direct medical costs and decreased productivity. All of these aspects together— 'quality-of-life', social, academic, and occupational impacts, along with direct and indirect costs, encompass the burden of disease of AD.
Atopic dermatitis has profound impacts on patient and family quality of life. A conservative estimate of the annual costs of atopic dermatitis in the United States is $5.3 billion (in 2015 USD). People with atopic dermatitis may even have to change their occupation because of their skin disease. Research gaps include quality of life assessments outside of tertiary care centers, impacts on partners and families of adult patients, and updated comprehensive cost estimates.

One new treatment has been introduced which has given a limited degree of positive data. This is a systemically administered antibody to the IL-4/13 receptor called Dupilumab blocking the action of IL-4 and IL-13. Not unexpectedly, for a molecule that totally abrogates Th2 antibody-associated immunity, it has significant side effects and causes blepharitis, conjunctivitis, eosinophilia, eye pruritus, headaches and oral herpes infections. In a recent French trial (*Faiz S, Giovannelli J, Podevin C, et al; Groupe de Recherche sur l'Eczéma 7. aTopique (GREAT), France. Effectiveness and safety of dupilumab in a real-life French multicenter adult cohort. J Am Acad Dermatol. 2019;81:143-151*), however, the incidence of conjunctivitis was much higher (38.2% vs 8%) than expected. Non-infectious ophthalmologic adverse effects resulted in discontinuation of Dupilumab in 10 patients. The other notable adverse event in this real-world trial was hypereosinophilia. Whereas the previous clinical trials showed transient eosinophilia in < 2% of patients, the incidence was five fold higher in this trial (9.5%). There is obviously a need for more directed therapy.

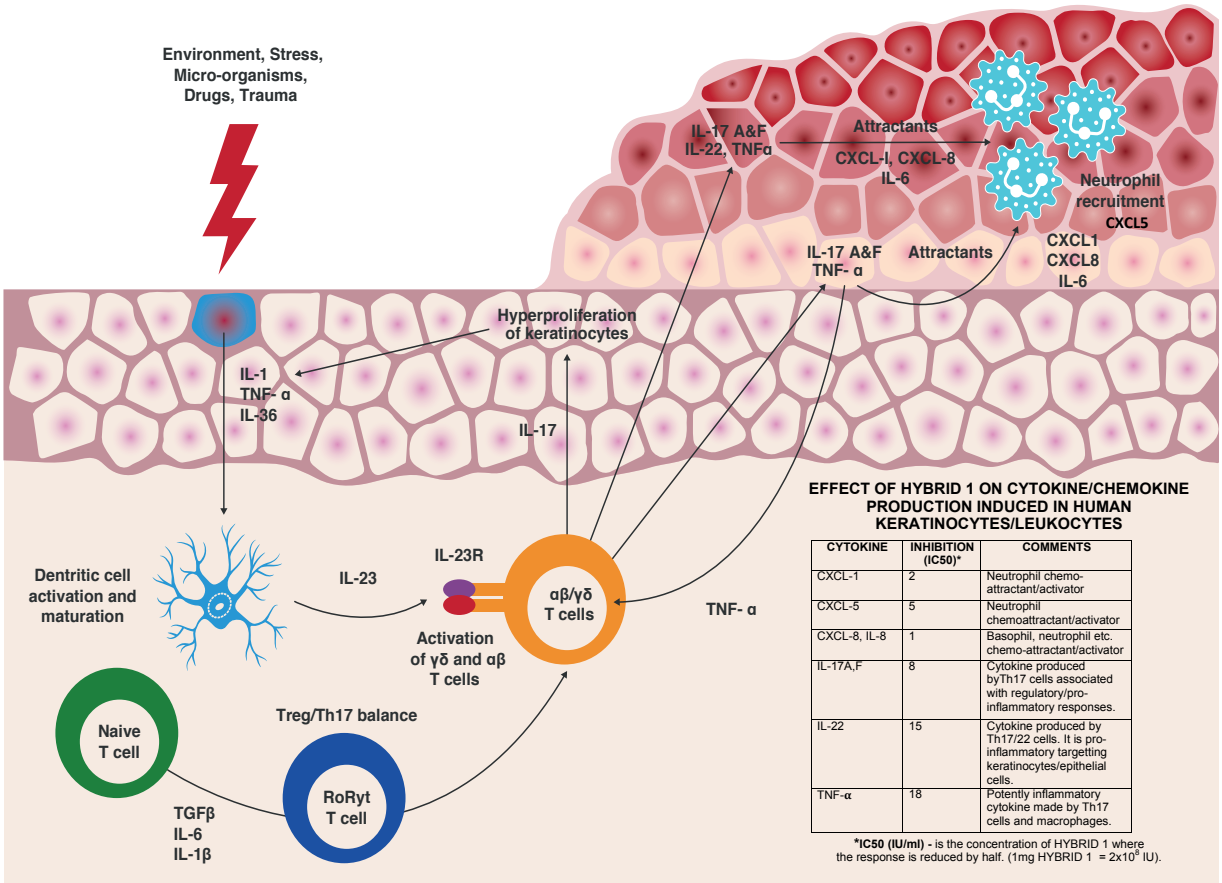
Alpha-interferon is recognized to be valuable in the treatment of certain cancers and viral infections but only Subtypes 2a,b,c have ever been thoroughly investigated and utilized clinically. We have studied several of the other subtypes and found that they can possess different and enhanced immunoregulatory properties, exhibit different immunological properties in different tissues(e.g. the skin) and that the properties they display are highly concentration dependent over a very wide concentration range.
In particular have we have identified one subtype that possess many of the features required for the treatment of AD/Psoriasis. Unfortunately it also possesses some features of potentially unwanted innate cell activation so we, therefore, decided to build 'synthetic' alpha-interferons based on the sequences of more than one subtype. This we have now achieved and we have hybrid interferon molecules with unique properties. One of these molecules has properties making it potentially suitable for the topical treatment of Psoriasis or AD. This is called HYBRID 1 and it has been patented in the EU, USA and Canada.
Finally, recombinant production of HYBRID 1 is extremely cost effective as it uses E.coli to obtain a very high yield. Also initial development of a topical formulation for HYBRID 1 have indicated no outstanding issues from the formulation company, Aptuit

Ltd, UK.

It can be seen in FIGURE 2 that Psoriasis is basically a one pathway disorder through IL-17 – so is highly susceptible to therapy with 'monospecific' monoclonal antibodies - nevertheless it still involves a wide range of interleukins and chemokines which are the 'final' effector molecules of the disease and these can be targeted directly to alleviate many of the symptoms, especially in the skin. AD initiation/persistence on the other hand is multifactorial in nature and in order to gain adequate control several of the pathways should be moderated simultaneously. We believe that our HYBRID 1 molecule has the multi-specific capacity to modulate both the interleukin and chemokine pathways involved in both disorders, especially via a topical route of administration.

FIGURE 3 below shows the Adaptive and Innate pathways that are now recognised to be activated, in the skin, in Psoriasis. Following the triggering events, dendritic cells release IL-23 which then activates Th17 cells. These Th17 cells maybe **α**/β, γ/**δ** or innate.

FIGURE 3



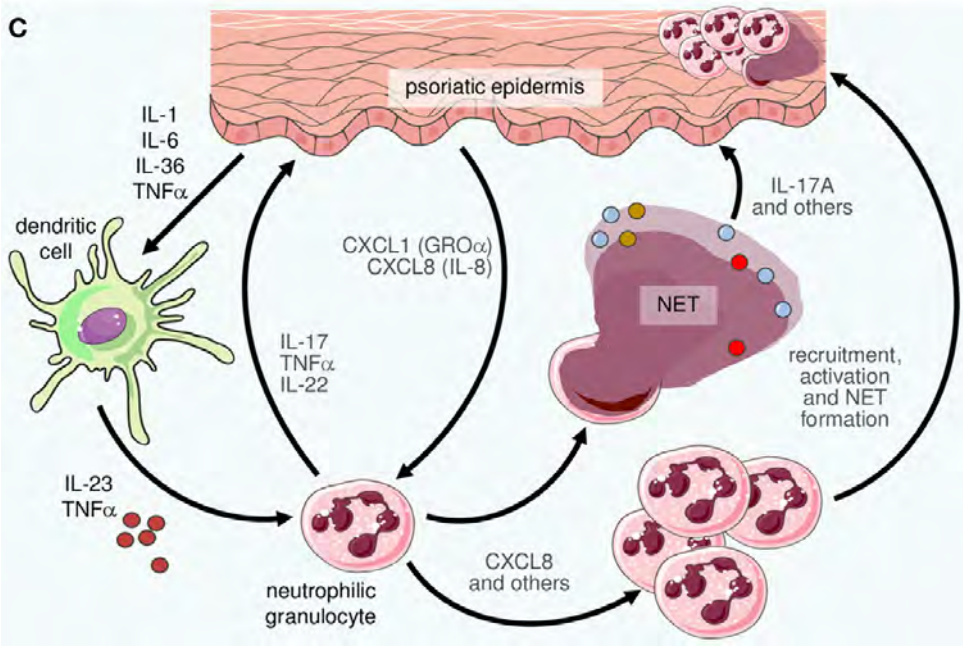
EFFECT OF HYBRID 1 ON CYTOKINE/CHEMOKINE PRODUCTION INDUCED IN HUMAN KERATINOCYTES/LEUKOCYTES

CYTOKINE	INHIBITION (IC50)*	COMMENTS
CXCL-1	2	Neutrophil chemo-attractant/activator
CXCL-5	5	Neutrophil chemoattractant/activator
CXCL-8, IL-8	1	Basophil, neutrophil etc. chemo-attractant/activator
IL-17A,F	8	Cytokine produced byTh17 cells associated with regulatory/pro-inflammatory responses.
IL-22	15	Cytokine produced by Th17/22 cells. It is pro-inflammatory targetting keratinocytes/epithelial cells.
TNF-α	18	Potently inflammatory cytokine made by Th17 cells and macrophages.

***IC50 (IU/ml) -** is the concentration of HYBRID 1 where the response is reduced by half. (1mg HYBRID 1 = $2x10^6$ IU).

The outcome of this activation is that the Th17 cell releases the cytokines, IL-17A/F, TNF-α and IL-22 (the IL-22 may also be derived from Th22 cells). IL-17A also interacts with macrophages in the dermis to cause a large enhancement in the synthesis of TNF-α from these cells. These cytokines, especially the TNF-α, then interact with the keratinocyte layer causing the induction of a number of chemokines, especially CXCL1, 5, and 8. Such molecules are highly chemotactic for neutrophils and basophils/mast cells and the cells concentrate in the keratinocyte layer before releasing the contents of their granules, which contain acid hydrolases, defensins, collagenases, histamine, leukotrienes etc – molecules that are involved in plaque formation in psoriasis. These activities are expanded in the following FIGURE 4 where the neutrophils/granulocytes form an 'extracellular net' of DNA within the epidermis to accentuate their activity.

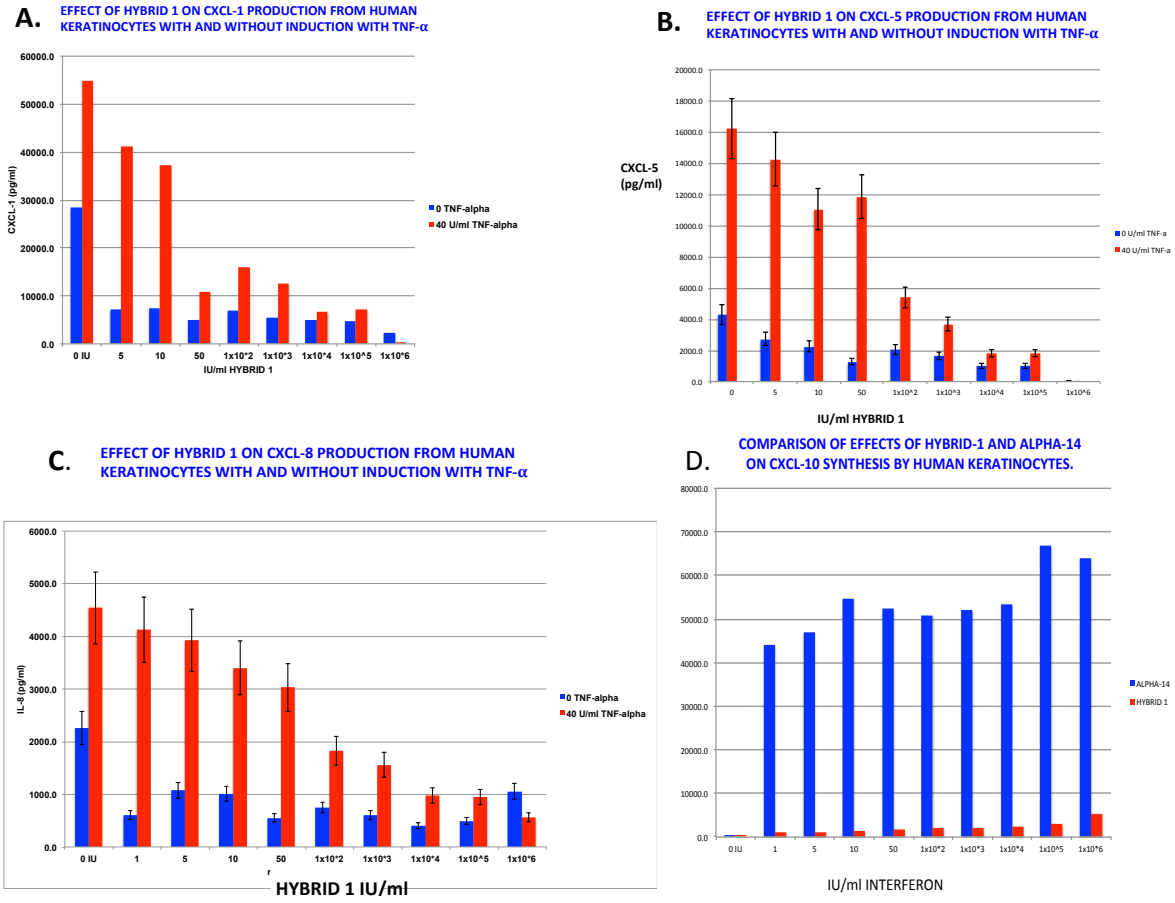
FIGURE 4



The effects of HYBRID 1 on all of these cytokines and chemokines are shown in the insert to FIGURE 3 – production of IL17A/F, IL-22, TNF-α from leucocytes and CXCL1, 5, 8 (secreted by TNF-α induced human keratinocytes are ALL inhibited at very low doses of HYBRID 1.
IC_{50}s are all less than 18IU/ml, where 1mg HYBRID $2x10^8$IU.

IL-1, 23, 36,TGFβ are not affected at all, which indicates the high level of specificity of HYBRID 1, especially when compared with the non-specific inhibition of cytokines and associated molecules by topical corticosteroids and JAK inhibitors.

FIGURE 5. The effects of a concentration range of HYBRID 1 on the human keratinocyte synthesis of CXCL-1,5,8,10.



The Figure A,B,C shows the potency of Hybrid 1 towards the production of the neutrophil/basophil chemokines CXCL-1,5,8. D indicates a comparison between HYBRID 1(red bars) and Interferon Subtype Alpha-14 (blue bars). CXCL-10 is a highly chemotactic activator of NK cells and such is NOT required for the treatment of Psoriasis/AD. It can be seen that HYBRID 1 has a significantly reduced capacity to induce the synthesis of CXCL-10.

In addition IL-6 is also suppressed which has implications for the source of Th17 cells (FIGURE 3 and 6). Evidence indicates that in the absence of IL-6 Treg cells are formed instead of Th17 lymphocytes. Thus we have additional control over the IL-23/IL-17 pathway as Treg cells do not make 'IL-17' cytokines. IL-6 is also involved in the generation of 'itch' in AD.

FIGURE 6 shows a typical dose response for Hybrid 1 suppression of IL-6 production in a human whole blood assay.

FIGURE 6

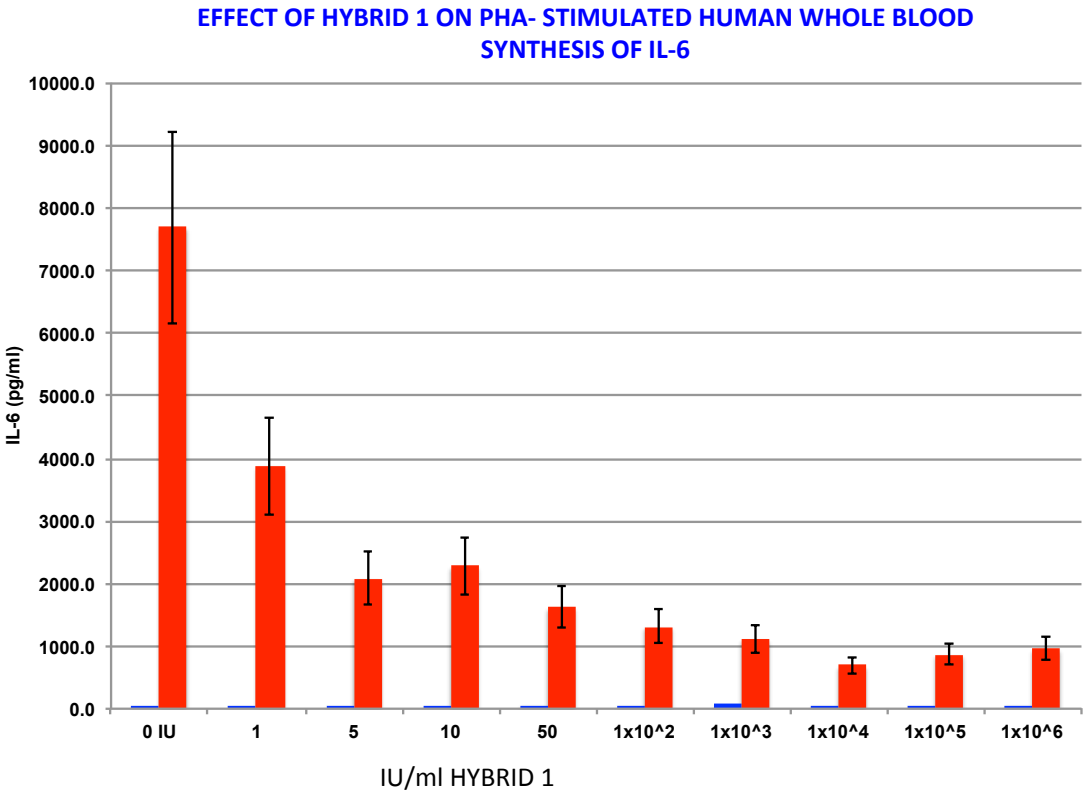


FIGURE 7. SYNOPSIS of 400 INTERLEUKINS, CHEMOKINES and PROTEIN MARKER ESTIMATIONS by RAYBIOTECH QUANTIBODY SYSTEM following HYBRID 1 TREATMENT of HUMAN MONONUCLEAR CELLS

ANALYTE	FOLD NUMBER CHANGE OF UNSTIMULATED HYBRID 1-TREATED CELLS	FOLD NUMBER CHANGE OF STIMULATED HYBRID 1-TREATED CELLS*
MOLECULE	HYBRID 1	HYBRID 1
INTERLEUKINS		
IL-1a	0	1
IL-1b	0	1
IL-2	0	+7
IL-3	0	-11
IL-5	0	-420
IL-6	-20	1
IL-8	1	1
IL-10	0	1
IL-12p40	0	0
IL-12p70	0	+11
IL-13	0	-5
IL-17	1	-40
IL-22	1	-25
G-CSF	-1500	1
All other interleukins to IL-35 - no effect.		
CD MARKERS		
CD23	-23	-850
CD97	1	-5
CHEMOKINES		
CXCL1	-780	-340
CXCL5	-6	-32
CXCL10	+4	1
CCL1	0	-25
CCL7	1	-140
CCL16	0	-200
CCL20	-65	1
TIE1	-160	-8
TREM 1	+5	1
ACE 2	+12	+7

0 = no analyte detected: 1 = analyte present but no effect of alpha subtype. Red – positive effect of alpha-subtype: blue – negative effect of alpha-subtype interferon.
* Cells stimulated with PHA.

FIGURE 7 gives details of significant changes in 400 parameters measured by an array ELISA system. The effects of HYBRID 1are shown here on human peripheral blood leucocytes in a resting state and stimulated with phytohaemagglutinin (PHA). An immediate observation is that only a limited number of cytokines/CD markers/ chemokines showed a significant response, indicating that HYBRID1 has discreet immunological targets.
Figure 1 indicates that AD is characterised by an over active Th2 response with IL-4 and IL-13 together with the IL-17 and 22 pathways also contributing to the disorder – IL-5 and GCSF are also known to play an important role too. It can be seen readily that HYBRID 1 has the capacity to reverse the majority of these factors and thus should provide a more multi-faceted approach to the treatment of AD than a specific monoclonal antibody like Dupilumab. In addition, although HYBRID 1 stimulates Th1 immunity at the expense of Th2 through IL-12p70 it does not Inhibit IL-4 and, therefore, should not overtly block Th2 immunity like Dupilumab, leaving the patient open to bacterial infection.
HYBRID 1 also inhibits CD23, the low affinity IgE receptor on basophils/mast cells by 850 fold and will, therefore, stop the release of histamine, prostaglandins, leukotrienes etc. and thus inhibit allergic reactions. Finally a number of important chemokines which act as chemotactic agents for neutrophils, basophils and monocytes were also highly suppressed. This means that cells would not be attracted to the site of the AD and, therefore, the inflammatory contents of their granules would not be released and contribute to the disease.

Additional work has been carried out using biopsies from normal human skin and human keratinocytes grown as monolayers. FIGURE 3 depicts what we have been found in a psoriasis model and many of the cytokines/chemokines are highly applicable to the adverse immune responses seen in AD. It should be noted that HYBRID 1 inhibits all of the cytokine and chemokines shown to arise after the Th17 cell. Specifically, IL-17A/F, IL-22, TNF-α, IL-6, IL-8, CXCL-1, CXCL-5. These are the molecules that alter the physiology of the keratinocyte layer, attract granulocytes of all types and cause the release of noxious chemicals/enzymes/small molecules to damage the layer.

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