

Progress report for the WAO short-term fellowship entitled "Is Fc-epsilon-RI an antigen uptake/presentation receptor in the intestinal mucosa involved in the initiation of allergic immune responses in the gastro-intestinal tract? an *in vitro* approach"

Background and Objective:

Allergy is a common problem in the Western world. Epidemiologic studies have shown that particularly the number of children suffering from allergies is increasing significantly from year to year. Recently, food allergic reactions in children are of increasing concern in the United States. Approximately one fourth of all American households faces restrictions in their diet due to a family member suffering from food allergies.

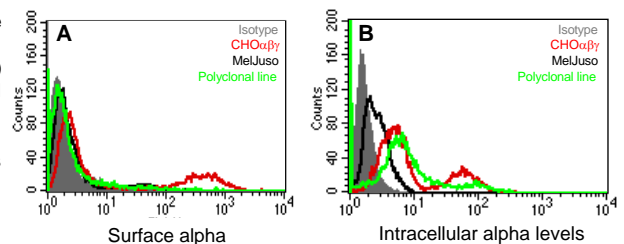
We hypothesize that Fc ϵ RI, the high affinity IgE receptor, may function as an antigen uptake/presentation receptor on DCs in the intestinal mucosa in a way that affects intestinal homeostasis and acute/chronic immune responses in children. We further postulate that the importance of Fc ϵ RI for IgE-mediated immune events at mucosal surfaces is underrated due to the fact that humans express Fc ϵ RI isoforms on antigen presenting cells that do not exist in rodents and are so far not studied in cell line models. The objective of the fellowship was to learn fundamental techniques required for IgE-loading and antigen uptake studies with a model of a human antigen presenting cell line expressing Fc ϵ RI. This cell line was established in the lab and is an excellent tool to understand the cell biologic events underlying Fc ϵ RI-mediated antigen presentation.

Results:

1. Surface expression of in Fc ϵ RI in MelJuso cells that stably express the IgE-binding alpha chain.

In the first set of experiments we compared expression levels of PCalphaHA (polyclonal MelJuso cell line that expresses Fc ϵ RI α) with an established CHO α β γ line by FACS (Figure 1). In contrast to the CHO α β γ line, PCalphaHA does not express significant levels of receptor at the surface (Figure 1A). We

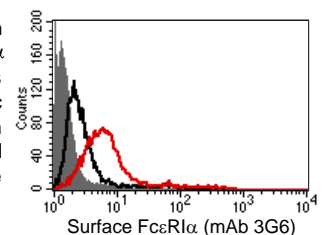
Figure 1: Expression levels of Fc ϵ RI α in the polyclonal MelJuso cell line PCalphaHA. Comparison with a CHO α β γ line by FACS. (A) Surface expression on CHO α β γ line, (red) and PCalphaHA (green). (B) intracellular expression of the alpha chain. Staining was performed after fixation and permeabilization of the cells. X-axis shows the expression levels of the alpha protein as determined by its reactivity with the specific mAb 3G6. Isotype control is given in grey.



were however able to detect significant intracellular expression of Fc ϵ RI α (Figure 1B). The most likely explanation for our finding is that the base line gamma chain expression of MelJuso is not sufficient for robust surface display of Fc ϵ RI. The finding would then be in line with a low steady state surface expression of CD64 by MelJuso cells (data not shown). Alternatively, surface expressed Fc ϵ RI α receptor might be quickly internalized and degraded in the absence of the ligand. The experiment was repeated with a single cell clone of this cell line. Additionally, a stimulation experiment with INF γ was performed (15 ng/ml, 16h) to induce expression of the common gamma chain. This experiment shows that indeed more receptor routes to the cell surface when we induce higher expression levels of gamma (Figure 2).

Conclusion: We are able to express Fc ϵ RI α at the surface of our cell line and thus can use these cells as a model for IgE-mediated antigen uptake.

Figure 2: Cell Surface expression levels of Fc ϵ RI α in MelJuso Fc ϵ RI α HA clone B5. Surface staining was analyzed by binding of specific antibodies. Isotype control is given in grey. Black line shows untreated cells, red line shows cells that were treated with INF γ for 16 h (15 ng/ml).



2. Antigen uptake in Fc ϵ RI in MelJuso cells.

We thus proposed to use MelJuso cells for our trafficking experiments. MelJuso is a melanoma-derived cell line that displays DC/macrophage characteristics and is established as a model of non-professional antigen presenting cells. A series of experiments performed during the fellowship showed that MelJuso cells do not up-regulate MHC class I and MHC class II surface expression upon LPS treatment a standard stimulus for the induction of DC maturation (data not shown). CD64, the high affinity receptor for IgG, is upregulated after LPS stimulation (data not shown). The two other human immunoglobulin family IgG receptors, CD32 and CD16, as well as the low affinity receptor for IgE, CD23, are absent on resting as

well as stimulated MelJuso cells (data not shown). Dextran uptake experiments show that MelJuso cells readily perform fluid phase antigen uptake (Figure 3). Upon LPS encounter MelJuso cells rapidly shut down their ability to perform fluid phase antigen uptake (data not shown), which is considered a hallmark of DC activation. Based on these results we are convinced that we can use the cell line as a model for antigen uptake and IgE-antigen trafficking experiments.

Conclusions: Meljuso show antigen uptake characteristics that match the published literature for classical APCs. Our results confirm the choice of this cell line as a model of IgE-mediated antigen presentation.

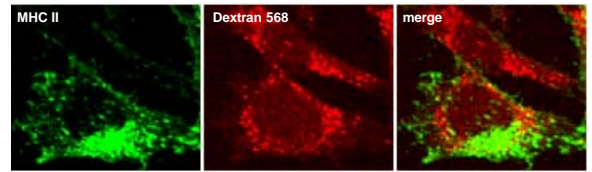


Figure 3: Antigen uptake by unstimulated MelJuso cells. Within 15 min Dextran568 as a fluid phase marker (second panel) can be detected in the cells. MHC class II was visualized with mAb MEM 138 and an Alexa 488 coupled anti-mouse second step reagent (first panel). The antigen did reach some but yet not all loading compartments at this time point (merge, third panel).

Summary, future directions and perspective:

The goal of this fellowship was to acquire technical knowledge in generating a model of a human antigen presenting cell line expressing $Fc\epsilon RI$ *in vitro*. This goal is met and even extended. The candidate also had the possibility to work with models of antigen uptake. Based on the experimental skills and the understanding for this multimeric receptor obtained during this fellowship, the candidate was recently able to demonstrate the expression of $Fc\epsilon RI$ in the human GI tract for the first time (data not shown), indicating *in vivo* relevance of this receptor. These new findings encouraged us to extend our collaboration to further develop our model of $Fc\epsilon RI$ -expressing antigen presenting cells, with the final goal to perform antigen-uptake and -trafficking, as well as functional antigen presentation studies in T-cell systems and intestinal epithelial cell models.

The exciting new data that were generated since the fellowship in Boston show how important such fellowships are for scientific exchange and collaboration. We are more than excited about these new findings and are grateful to the WAO for their support.