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Immunological Immaturity of the Neonate, Protein Kinase C Zeta and Allergy

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Introduction

The epidemic rise in allergic disease over the last 40 years has made prominant the urgent need to identify the developmental pathways that lead to disease. As potentially more effective interventions for allergy prevention are developed, such as primary sublingual immunotherapy, it will become crucial to accurately identify children who will develop disease. But there are currently no biological markers that have any established predictive value [1]. Cord blood IgE, the only neonatal marker to be extensively evaluated as a likely predictor, has been shown to be unreliable in this context [1,2]. A "family history" remains the only crude predictor of allergic disease in use [2-4].

Evidence of immunological markers of allergic propensity in the neonatal period has been presented. The development of allergic disease is frequently preceded by immunologic differences that are already evident in the neonatal period [5-10]. The most consistently observed difference has been reduced production of Interferon gamma (IFNγ by T cells [5-10], suggesting that relative T cell immaturity may be an important contributor to disease development. Although it is not clear whether this T cell "defect" is primary or secondary to antigen presenting cell immaturity, findings support the presence of intrinsic differences in T cells from neonates with subsequent allergic disease. Despite the relationships between Th1 IFNγ production and allergy risk [5-10], this has not been a reliable predictive marker because it, along with other functional measures, relies on inherently variable in vitro culturing systems with potent polyclonal stimulants that potentially distort patterns of response [1].

Early life exposures can modify T cell immune function. Preliminary studies also suggest that maternal environmental exposures such as infection [11], maternal diet [12,13] and smoking [13,14] can modify neonatal T cell function, although the mechanisms are not clear. With rising disease rates, there is a continuing urgency to identify the pathways involved and to explore and monitor the effects of early interventions that could favourably influence the functional development of T cell responses and prevent allergic disease. Recently studies provided the first evidence that an intervention designed to reduce allergic disease had a significant effect on the protein kinase C (PKC) pathway [15].

Immunological Immaturity of the Neonate

Neonatal T cells are immature and lack or are poor in a number of T cell responses compared to cells from older babies and adults. In a study on the responses of T cells to the mitogen phytohaemagglutinin (PHA) in neonates versus adult blood in terms of IFNγ production, cells from term babies produced only about 10% of the amount of IFNγ produced by adult T cells. This production was compromised even more if the babies were preterm (unpublished data). This deficiency is reflected in an inability to stimulate the downstream intracellular molecules, the mitogen activated protein (MAP) kinases such as ERK and JNK [16]. In a biochemical study examining the intracellular signaling in neonatal T cells it became apparent that some of this immaturity was a result of an inadequacy in the PKC-MAP kinases signaling axis. These studies demonstrated that not only were the MAP kinase not activated after the T cell receptor was engaged but also when agonists acting on downstream signaling molecules/pathways of the receptor, namely PKC and calcium mobilisation stimulated by PMA and A23187 respectively [16].

However it became evident in these studies that examining the levels of the various PKC isozyme expressed by the T cells some of these isozymes were reduced in neonatal T cells. Cord blood T cells were found to be deficient in expression of PKCβI, ε, θ, and ζ isotype [16]. Interesting as the T cells mature these is normalisation of these levels concomitant with an ability of T cells to show normal immunological responses [17].

Protein Kinase C and Allergic Predisposition

The 'fingerprint' pattern of neonatal T cell PKC isozyme expression significantly predicts subsequent allergic disease [15]. Moreover intervention designed to prevent allergic disease, maternal dietary supplementation with fish oil, significantly modified the expression of neonatal PKC [15]. These novel findings provide a strong basis for investigating this signaling protein further as a potential neonatal screening test for allergy prediction and potential target for disease prevention.

Evidence suggests that reduced neonatal PKCζ expression is associated with allergy outcomes. Reduced responsiveness of neonatal T lymphocytes is associated with reduced expression of several PKC isozymes and reduced ability to activate MAP kinases [16]. MAP kinases differentially regulate Th1 from Th2 cytokine production [18-21]. There is large individual variation in neonatal T cell expression in PKC isozymes, and it is therefore logical to surmise that these variations in PKC isozyme expression lead to corresponding differences in maturation characteristics, patterns of cytokine responses and disease susceptibility.

Neonatal T cell PKC isozymes are differentially expressed, with

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PKCζ varying inversely with most other isozymes [15-17]. Examination of these PKC isozymes in relationship to allergic outcomes in a cohort of 74 "high risk" children in a previously recruited cohort, showed that increasing levels of PKCζ expression had a "protective" relationship with allergic disease and sensitisation at both 1 year and 2.5 years of age [17]. PKCζ appeared best at predicting food allergy with significantly lower levels in neonates who developed a positive skin prick testing (SPT) to egg. Only SPT positive and not SPT negative dermatitis was associated with PKCζ expression, suggesting an association with atopy rather than dermatitis *per se*. Similarly, only persistent wheeze, and not early transient wheeze, was associated with PKCζ expression. Thus the variable inadequacy in PKC kinase signaling may play a role in the variations in neonatal T cell phenotype and subsequent disease risk.

Neonatal T Cell PKCζ and IFNγ Production

While neonatal PKCζ was found to be significantly correlated with Th1 IFNγ production, it was a more powerful predictor of allergic disease than the cytokine. Neonatal polyclonal IFNγ responses were lower in children with allergic disease by 1 year [5-10,17]. Interestingly, these were significantly correlated with PKCζ expression [17]. PKCζ was the strongest independent predictor of allergic disease in this population. Specifically, using multivariate logistic regression with these parameters as predictor variables, only PKCζ significantly predicted allergic outcomes but not the neonatal T cell IFNγ production. This effect was independent of other exposures or interventions [17].

Antenatal Intervention to Modify PKCζ

The ultimate in this new development would be to modify expression of PKCζ in neonatal T cells via maternal nutrient supplementation. Maternal supplementation with n-3 polyunsaturated fatty acids (fish oil) in pregnancy modifies neonatal immune function. The predominant effects were seen on T cell responses [12,22]. It was speculated that n-3 long chain polyunsaturated fatty acids (LCPUFA) could promote changes in developing patterns of T cell responses by influencing the relative pattern of PKC isozyme expression. Interestingly the suppression of neonatal T cell cytokine responses by fish oil [12] was associated with changes in PKC expression. Notably [17], while most isozymes were down-regulated by fish oil, PKCζ was significantly higher in the fish oil group [17]. As PKCζ was shown to be an independent predictor of reduced subsequent allergic disease, this could present an important pathway through which antenatal n-3 LCPUFA could have clinical effects in reducing allergic disease [12]. It is tempting to speculate that interventions during an "early critical window" of development can alter the risk of subsequent sensitization and disease. At this stage the timing of this "critical window" is not clear, although perinatal differences in immunity of allergic individuals indicate that this is likely to be early in development [9,23,24]. It is therefore logical that to be most effective, preventive interventions also need to be targeted early.

PKCζ as a Screening Test

The generated ROC (receiver operating characteristic) curves to determine optimal "cut-off" levels for predicting allergic disease demonstrated that PKCζ expression below 62.3% (% of normal levels) predicted allergic disease with a sensitivity of 80% and a specificity of 63% (and accuracy of 71%; the number of children correctly classified) in this "high risk" population [17]. These results were reproduced in a second cohort study in which the cumbersome western blotting analysis of PKCζ was replaced with a flow cytometry test involving intracellular staining of the protein [16]. This suggests that PKC is superior to any other biological marker assessed in this context, including cord blood IgE, which has a reported sensitivity of only 26-47% [2-4,25]. Although other parameters have been examined previously, including eosinophil levels, histamine reactions and leukocyte phosphodiesterase levels, none had an accuracy of more than 58% [26].

Concluding Remarks

The finding that neonatal T cell PKCζ expression is lower in allergic children, and that this isozyme is also significantly enhanced by increased membrane n-3 LCPUFA content, opens new horizons in developmental immunology in which levels of intracellular signaling molecules may be amenable as markers of T cell development. Variations in these may be relevant to functional immune phenotype and disease development. This not only provides further evidence that environmental interventions can significantly modify neonatal T cell function, but also suggests that PKCζ should be explored further as a marker of disease risk. As disease prevention strategies become more refined "risk markers" will be of increasing importance to target interventions and to assess their effects.

Reduced neonatal PKCζ expression in subsequently allergic children was detected in resting cells without the need for stimulation or culture. Accordingly, the "predictive" effect of PKCζ is far greater than IFNγ Th1 responses, which depend in inherently variable functional *in vitro* culturing systems. As there are no reliable early markers to predict allergic disease, the PKCζ marker should be explored further in this context, particularly as this approach avoids *in vitro* manipulations and could be more readily standardised. In addition through a better understanding of these pathways we may improve avenues for more targeted treatment and prevention of allergic diseases.

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