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Summary

The meaning of eosinophilic cationic protein (ECP) determinations in patients with allergic asthma. Jansen RW, Maas L van de, Vermes I. Ned Tijdschr Klin Chem 1996; 21: 211-213. Bronchial hyperreactivity and eosinophilic inflammation play a pivotal role in the pathogenesis of allergic asthma. In recent years much attention was focussed on the use of serum ECP measurements to quantitate the inflammatory component of the asthmatic process in order to monitor the corticosteroid therapy. This paper deals with the rationale of serum ECP measurements and shows the results of a clinical trial in which the effect of corticoid therapy on serum ECP and lung function of allergic asthmatic patients were investigated.

Keywords: allergic asthma; eosinophilic cationic protein (ECP); corticosteroid therapy

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IgE, eosinophils and mast cells in helminth infections

M. YAZDANBAKHSH

Helminth parasites are a cause of extensive morbidity in most tropical countries. Considering that over one billion people are infected, these parasites are a serious burden on human health. Generally, helminths display a complex life cycle with several developmental stages that migrate through or reside in distinct organs. Intestinal helminths might be restricted to the gut, such as *Trichuris* or *Enterobius* or have tissue migrating phase such as *Ascaris* and *Strongyloides*. Other helminths of importance to humans are schistosomes that reside in the abdominal veins and filariae which nest themselves in the lymphatics (*Brugia malayi* or *Wuchereria bancrofti*), the skin (*Onchocerca volvulus*) or migrate through the connective tissues throughout the body (*Loa loa*). The hallmark of helminth infections, in immunological terms, is elevated IgE and eosinophilia. Total IgE levels can exceed those seen in asthmatic or atopic dermatitis patients (figure 1), and eosinophil numbers can range from moderate to extremely high (over $40 \cdot 10^9/l$). Intestinal helminth infections are associated with mast cell hyperplasia, as reported in experimental infections (1); colonoscopy of infected individuals has confirmed the presence of mast cells at sites of inflammation (2). Histological examination of the tissues in elephantiasis limbs (caused by either *Wuchereria bancrofti* or *Brugia malayi*) has shown the presence of mast cells in the inflammatory mass around degenerating worms. The question that has

been asked over and over again is whether IgE, eosinophils and mast cells that are closely associated with helminth infections play a role in protective immune responses.

Human studies

Chronic infections

It is now a well accepted concept that T cell cytokine profiles can be classified into two opposing poles of a spectrum; TH1 cells that release $IFN\gamma$ and are key regulators of cell mediated immunity and TH2 cells that elaborate IL-4 and IL-5, cytokines essential for IgE and eosinophilia respectively (3). In keeping with the high IgE and elevated eosinophils observed in helminth infections, there is an abundance of T cells capable of releasing IL-4 and IL-5 in patients with chronic schistosomiasis or filariasis (4,5). Thus at the polyclonal level both IL-4 and IL-5 are associated with increased IgE and eosinophils. Next, the parasite specific responses will be discussed.

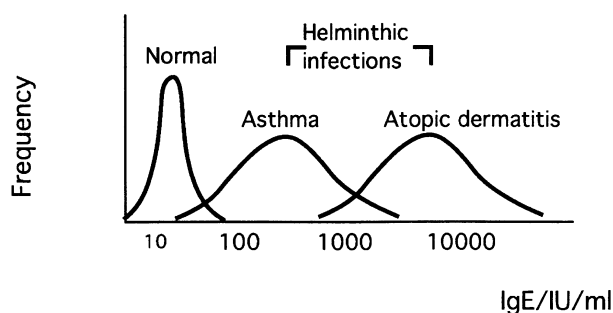


Figure 1. Serum total IgE levels in allergic patients and helminth-infected individuals.

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As helminth infections are chronic in nature, and the worms do not multiply within their mammalian hosts, they must have evolved mechanisms to evade the immune response and to ensure their long term survival. In humans, the comparison of immune responses between infected and non-infected (but exposed) individuals or before and after removal of parasites by chemotherapy has been instructive to characterize the immune responses that are important in combating infection and to understand the modulation of host immune reactions by the parasite. In schistosomiasis, epidemiological studies have provided evidence for involvement of TH2 type responses in resistance to re-infection. In elegant studies carried out in areas where *S. mansoni* and *S. haematobium* are endemic, it was shown that individuals resistant to re-infection after chemotherapy were those with high IgE (6,7) and elevated eosinophils (figure 2) (8). More recently the cytokine release profiles have been examined in subjects susceptible or resistant to re-infection. Interestingly, not only IL-5 but also IFN γ correlates with resistance to reinfection (9). Comparing cytokine release before and after chemotherapy has revealed that infection with *S. haematobium* is associated with suppressed IL-4 and IFN γ (10), indicating that both TH2 and TH1 cytokines may play a role in resistance to infection.

In filariasis, no re-infection studies have been carried out so far. However, individuals harbouring high worm burdens show profound antigen specific T cell hyporesponsiveness and release low levels of IL-5 and IFN γ compared to uninfected but exposed subjects (11, unpublished results). Moreover, reduction of worm burden by chemotherapy in filariasis is associated with increased cell proliferation and IFN γ production (12) whereas IL-4 is unaffected (IL-5 has not been measured). At the antibody level, antifilarial IgG4 responses are stimulated by the presence of worms and decline rapidly following chemotherapy whereas IgE does not follow this pattern (13). One interesting spin off from these studies has been the development of a diagnostic test; elevated specific IgG4 in lymphatic filariasis has been used to identify infected individuals or assess prevalence in endemic areas (14). It can be concluded that favourable conditions for long term survival of filarial worms is low parasite specific TH1 (typified by proliferation and IFN γ), low antigen driven IL-5, expansion of IL-4, high specific IgG4 and relatively low IgE directed to filarial antigens. For schistosomiasis, low specific IL-4, low antigen driven IFN γ and low specific IgE appear to be associated with worm survival. Thus although at polyclonal level IL-4 and IgE are highly expanded in helminth infections, the specific IgE levels are kept at low levels.

There are few data on the protective mucosal immune responses to intestinal helminths in human beings. In one study of children with *Trichuris dysentery*, high levels of mast cells and IgE bearing cells were detected in the colon and these did not appear to cause appreciable parasite expulsion (2).

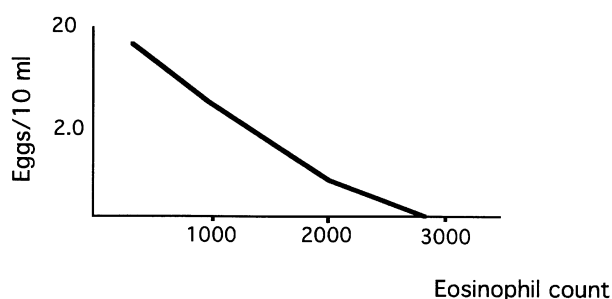


Figure 2. Relation between eosinophil levels and reinfection (Hagan et al. *Tras R Soc Trop Med Hyg* 1987; 81: 938).

Recent/acute infections

A different situation arises in infections that are not chronic in nature. Travellers from non-endemic regions entering endemic areas and experiencing infection for a short period of time, show both eosinophilia and elevated IgE (15). The levels of eosinophils are generally higher than that observed in chronic situations and although high levels of total IgE might be detected, there is often no appreciable IgE or IgG4 to parasite antigens. The cytokine release profiles also indicate that parasite antigens stimulate IFN γ production but no IL-4 production during the acute phase of the infection (unpublished results). How long exactly and how much exposure is required before the cytokine profile to parasite antigen in an immunologically naive individual shifts from TH1 to TH2 is not known.

Experimental infections

In murine models of schistosomiasis, TH2 responses do not mediate resistance to infection but appear to play a major role in granuloma formation. Indeed, in vaccinated mice, resistance to infection was abolished when mice were treated with neutralizing antibodies to IFN γ (16). Thus the situation is clearly very different from what has been reported for human schistosomiasis, where eosinophils and IgE, products of TH2, are correlated with resistance. With respect to filariasis, mice are refractory to the full development of filarial worms, but it is possible to transplant each life cycle stage and study the immune response elicited and assess parasite survival. Using this approach it has been shown that whereas infective stage L3 and adult worms stimulate TH2 type responses and downmodulate TH1, microfilariae elicit Th1 marked by high IFN γ (17). Although in filariasis Th2 responses may not be essential for resistance to initial infection by L3, as shown in IL-4 gene knock out mice (18), resistance to secondary infection appears to be TH2 cell mediated and depend on IL-4 and IL-5. It is still far from clear which responses are needed for killing adult filarial worms. Murine models of intestinal helminth infections have provided clear evidence for the importance of TH2 cells in parasite expulsion and administration of IFN γ enhances worm survival (19). Mastocytosis is essential for the expulsion of *Trichinella spiralis* and *Strongyloides ratti* (20) and although IL-3 and IL-4

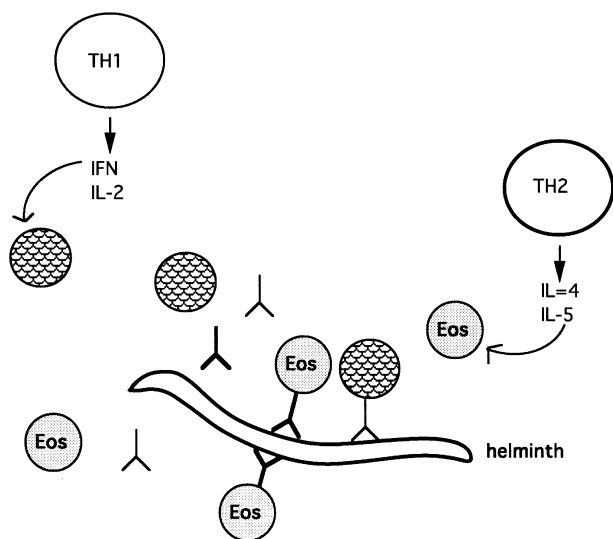


Figure 3. Schematic representation of how both TH1 and TH2 cell subsets can play a role in parasite killing. TH2 cells lead to increased IgE and eosinophilia. In man, IL-10 released by TH1 and TH2 cells can also play a role in antibody switch to IgG isotypes other than IgG4. TH1 cells release IFN γ , an important cytokine in enhancing the cytotoxic potential of granulocytes. Thus in combination, TH1 and TH2 cells can provide all elements necessary for effective parasite killing. To ensure long term infection, helminths must try to suppress pathways which are detrimental to their survival; IFN γ , IL-4, IL-5, IgE can all be points towards which immunosuppression is targeted to.

can regulate mastocytosis in the gut and thus the extent of expulsion, there is increasing evidence for an additional level of control by Stem Cell Factor (SCF) which also stimulates mast cell hyperplasia (20). In the intestinal helminth infection, it is not clear whether eosinophils and IgE have an essential role in worm expulsion.

So do IgE, eosinophils and mast cells play a role in controlling helminth infections??

It has been argued that the evolutionary advantage of the so called TH2 system lies in its role in the acquired immune response to parasitic helminths. However, there is currently no unifying mechanism underlying protective immune responses to infection or re-infection with helminths. In animal models, TH2 responses are critical for mediating immunity to intestinal helminths whereas TH1 type responses are necessary for vaccine induced protection in schistosomiasis. The data in human schistosomiasis contradict the findings in mice; in human schistosomiasis resistance appears to be associated with both elevated IgE and eosinophilia. As immunoepidemiological studies have indicated an association between resistance and increased IL-5 as well as IFN γ , it may be that with complex, multi-stage parasitic infections both TH1 and TH2 type responses are required to act. Indeed, it appears that each life cycle stage elicits a distinct type of polarized immune response. It is therefore not surprising that a coordinated interaction between opposing poles of a spectrum are needed for combating a full blown infection (figure 3).

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Chronische myeloïde leukemie: recente ontwikkelingen op het gebied van pathogenese, diagnostiek en behandeling met behulp van interferon of intensieve chemotherapie

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Pathogenese

Chronische myeloïde leukemie (CML) is een maligniteit uitgaande van hematopoïetische stamcellen, waarbij alle cellijnen kunnen zijn aangedaan. Kenmerkend is het reeds in de zestiger jaren beschreven afwijkende chromosoom 22 (Philadelphia chromosoom), welke afwijking het gevolg is van een reciproke translocatie tussen chromosoom 9 en 22. Hierbij ontstaat een chimeer oncogen (BCR-ABL), dat enerzijds bestaat uit delen van het c-ABL gen (afkomstig van chromosoom 9), dat codeert voor een tyrosine kinase enzym, en anderzijds uit een BCR-deel afkomstig van chromosoom 22. Indien het chimere oncogen experimenteel wordt ingebracht in het DNA van gezonde hematopoïetische stamcellen, ontstaat een op CML gelijkende proliferatie (1). Een oorzakelijk verband tussen het eiwitproduct van het BCR/ABL oncogen en CML is daarmee aannemelijk gemaakt. Dit chimere eiwit (P210) vertoont een verhoogde tyrosine-kinase activiteit, die waarschijnlijk verklaard wordt door een interactie van het enzym met het eiwitdeel waarvoor BCR codeert. Zowel de verhoogde tyrosine-kinase activiteit als een aantal andere eigenschappen vanuit het BCR-deel (2), waaronder activatie van de ras-pathway en van het oncogen c-myb, lijken verantwoordelijk voor de toegenomen proliferatie en differentiatie. De voor onbehandelde CML typische transformatie naar een blastaire fase gaat vaak gepaard met additionele afwijkingen aan oncogenen, waarbij ook mutaties of deleties aan het P53 proto-oncogen beschreven zijn (3).

Behandeling

Hydroxyureum en Interferon- α

De enige curatieve behandeling tot op heden is een beenmergtransplantatie met beenmerg van een geschikte (bij voorkeur verwante) allogene donor (4). Echter deze behandelingsvorm is slechts voor een minderheid ($\pm 20\%$) beschikbaar, enerzijds als gevolg van het ontbreken van een geschikte donor en anderzijds doordat de meeste patiënten bij presentatie boven de 50-60 jaar zijn. Patiënten die niet voor een allogene BMT in aanmerking komen, worden momenteel behandeld met hydroxyureum en/of alfa-interferon (IFN- α). Hydroxyureum verdient de voorkeur boven busulfan, daar een gunstig effect op de overleving in een gerandomiseerde studie kon worden aangetoond (5). Daarnaast is hydroxyureum gemakkelijker te doseren, ontstaan minder langdurige cytopenieën, en kan busulfan-gebruik soms gecompliceerd worden door longtoxiciteit.

Ook behandeling met IFN- α heeft een gunstig effect op de overleving. Inmiddels zijn de resultaten bekend van 3 grote gerandomiseerde studies, waar een duidelijk gunstig effect van IFN- α uit naar voren komt. In een Italiaanse studie (6) werden 332 patiënten in een 1:2 verhouding gerandomiseerd tussen IFN- α en conventionele chemotherapie bestaande uit of hydroxyureum of busulfan. Tweehonderd en achttien patiënten in 1^e chronische fase werden behandeld met IFN- α en 104 patiënten kregen conventionele chemotherapie. Het tijdsinterval vanaf randomisatie tot acceleratie/transformatie was significant langer in de interferon-groep (mediaan: >72 vs 45 maanden; $p < 0.001$) evenals de mediane overleving (72 vs 52 maanden; $p < 0.002$). Het gunstigste effect van interferon- α op de overleving was het meest uitgesproken in een kleine groep van patiënten (19%), die een complete dan wel "major" cytogenetische response bereikten (> 65% Philadelphia-negatieve metafasen). Deze kleine groep kon niet met de bekende prognos-

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