Interplay of host and infectious agents

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Wechselwirkung von Wirt und Pathogenen


Summary. In this group we would like to answer the question why people show a different response against certain pathogens. In many infections the course of the disease can range from asymptomatic carriage to the severest forms even death. In the past we have analysed candidate genes and their role in the course of malaria and could detect some polymorphisms influencing infectious diseases in the genes encoding NOS2, MBL2, IFNa, FCN2, and receptors for IFNg and IFNa. Having worked initially mainly on malaria we broadened our spectrum also to other infectious diseases like hepatitis B, leprosy, and schistosomiasis. Here we give a short overview about ongoing projects.

Key words: Malaria, leprosy, HBV, interferon, NK cells, host genetics.

Association of regulatory gene polymorphisms with Treg functional activity

A considerable component of our work is the investigation of human polymorphisms, which may underlie both susceptibility to infection and level of Treg expression. A number of loci most associated with Treg activity are studied, as well as those previously linked to helminth susceptibility. Loci tested include T-lymphocyte-associated protein-4, interleukin-10 receptor a and b, CD25, interleukins 4, 2, and 17, FoxP3, and tumor necrosis factor superfamily receptors.

Many of the loci selected above were first associated with inflammatory, non-infectious diseases such as diabetes and only recently have been linked to regulatory T cell frequency. For some, their role in infectious diseases has yet to be investigated, and our results will show whether these polymorphisms are maintained in the human population as a result of exposure to infectious parasites.

Despite antigen recognition, the failure of the immune system to clear parasites remains as a crucial barrier. As a part of parasite strategy to survive in a human host, parasites induce regulatory T cells which in turn suppress anti-parasite effector cells. This will lead to a modest immune response in humans during vaccination or recurrent infections as there will be insufficient effector cells to kill the parasites. Several polymorphisms at vital genes in the human immune system had been associated with immune pathologies.

We determine the DNA sequence of the genes mentioned above in a cohort of 40 adult healthy Africans, giving us the chance to find polymorphisms that are present in the population with 10% [1]. The promoter region is sequenced and additionally all exons. In addition to pre-described SNPs, we discovered novel SNPs in all the promoter regions of these genes. These novel SNPs were further validated by cloning and subsequent sequencing for their true polymorphisms. Further we will test the functionality of these regulatory gene polymorphisms with promoter constructs in vitro [2, 3].

Interaction of NK cells with P. falciparum – infected erythrocytes

Natural killer (NK) cells, which are effector lymphocytes of the immune system, play an important role in innate immune responses against Plasmodium falciparum. Apparently, the early production of interferon-gamma (IFN-γ) through cooperation between monocytes, macrophages and NK cells seems to be a very important factor to promote protective immunity to malaria. Although it was already shown that NK cells play a role in early P. falciparum infections, there are many unresolved questions about the in vivo functions of these cells. In this project we aim to investigate the events that occur in the event of contact between NK cells and P. falciparum iRBC. Our group works with NK cell lines as well as with freshly isolated NK cells to analyse the gene expression of this very special parasite/host interaction.
Two NK cell lines were tested for their NK cell features: the YT and NK92 cell lines. Both lines were purchased from DSMZ in Brunswick, Germany. Both lines were stimulated with either IL-12/18 and parasitized erythrocytes. Subsequently we analysed the expression of specific surface markers by cytometric means. After co-culture of 3D7-iRBCs with NKYT or NK92, the cells were tested for the formation of rosettes, release of IFN-γ and cell surface expression of activation markers. Although it was possible to detect some activation features in the NK cells, the data obtained in this study show that the cell lines are not properly activated by 3D7-iRBCs as NK cells within PBMCs would do. No rosettes were seen between iRBCs and the cells lines. NKYT cells lack expression of CD56 and appear to be constitutively activated, releasing high levels of IFN-γ regardless of iRBCs contact. NK92 cells expressed CD25 in response to iRBCs although CD69 was not up-regulated. These findings show that the NKYT cell line can not be considered as an adequate model for NK cell activation and that, although poorly activated, NK92 cells are able to respond to iRBCs stimulus.

As a pilot experiment we performed a co-incubation of NK92 cells with parasites. Here we used the parasite line FCR3-CSA2, a cell line that shows a distinct rosetting phenotype with NK cells. These rosettes consist of NK cells with attached parasitized red cells. When parasites were added to NK92 in a ratio of 10:1, no growth retardation was observed, however, when the number of parasitized cells was diminished to a ratio of 1:1, substantial growth retardation was observed. To investigate the nature of the growth retardation we performed microarray analysis on parasites, which had been in co-culture with NK92 cells for 14 hours. Parasites were separated from the NK92 cells by Ficoll density gradient and RNA was isolated from the cells. This RNA was then analysed on a P. falciparum-specific microarray purchased from Affymetrix. We compared the expression pattern of these parasites with parasites that were not cultivated with NK cells but purified on a density gradient. As base line expression RNA was isolated from parasites under normal culture conditions. To our surprise we found a higher number of genes downregulated by NK cells contact. After sorting the genes into functional groups we identified these genes as coding for merozoites surface antigens, protein processing, signal transduction, transcription, translation and transporters and others.

The fact that NK92 cells are able to kill parasites and that they are at least partly activated by parasites encourages us to rely on this model and it makes us confident that the parasite’s response is similar to the in vitro situation.

The serum proteins mannose-binding lectin and ficolin-2 in infectious diseases

Mannose-binding lectin (MBL) and ficolin-2 are serum proteins known to play a key role in pathogen recognition and clearance [4–7]. MBL targets invading microorganisms for phagocytosis and complement-mediated killing by binding to surface carbohydrates, such as N-acetyl-D-glucosamine, mannos, N-acetyl-mannosamine, fucose, and glucose [8]. Ficolin-2 binds to a different pathogen-associated molecular pattern (PAMPs), namely lipoteichoic acid and acetylated groups leading to the pathogen phagocytosis and activation of complement through the lectin pathway [9]. Polymorphisms in both genes result in a deficiency of the gene products. It has been shown by us that MBL binds to parasites such as Schistosoma mansoni, Plasmodium falciparum [10, 11] and that polymorphisms leading to MBL deficiency are involved in the course of a hepatitis B virus infection [12], autoimmune diseases like Crohn’s disease [13–15] and parasitic infections [16].

Interestingly, the same polymorphisms mediate a certain level of protection against mycobacterial infections with M. tuberculosis and M. leprae [17, 18]. The reason being that the bacteria, which are covered with MBL are more easily taken up by macrophages which is their preferred host cell.

The literature on ficolin-2 is far less extensive but in first analyses we could show that in rheumatic fever a functioning ficolin gene is of advantage [19, 20]. In other cohorts we study the polymorphisms of ficolin-2 also with schistosomiasis and malaria.

Virus and host factors in hepatitis B

Hepatitis B virus characterization in highly endemic countries is considered to be a crucial and vital step to tackle the resultant liver disease exp., Hepatocellular Carcinoma (HCC) and Liver Cirrhosis (LC). Hepatitis B genotypes play a pivotal role in liver infection prognosis in individuals who are prone to HCC and LC. Recent studies have inferred that there is an increased risk of developing HCC in patients with occult HBV. In a country like Nigeria, where HBV is highly endemic, little is known about the currently circulating HBV genotypes and on occult hepatitis. In this project we aim to investigate the prevalent HBV genotypes in Nigerian individuals and the effects of viral factors on liver disease outcome.

In recent work we investigated host polymorphisms in hepatitis B and focused on genes in the interferon alpha pathway since interferon alpha (IFN-α) is used as therapy against hepatitis B. We investigated whether the promoter polymorphisms in the gene for IFNa-2 gene which are known to cause variable expression levels in vitro, may influence the course of HBV infections. We found a deletion in the promoter, which occurs significantly more frequently in HBV-infected patients than in control individuals; 20% of the healthy, whereas 35% of the HBV-infected cohort carries this deletion (P<0.001). Reporter gene assays showed that a construct with the deletion had a lower level of transcription in comparison to the wild type (P=0.011) [21]. This reduction could explain the individually different interferon levels in humans and could also be one cause of susceptibility to hepatitis B. In a separate study we investigated this mutation in five other populations (199 Central Africans, 265 Brazilians, 108 Kaingang, and 98 Guarani) and could detect this variant in all populations. The presence of the deletion did not affect the course of a malarial infection but the positivity rate of HCV [22].

We also have evaluated the impact of two variants of the IFNAR1 gene on the outcome of HBV infection, which have been shown to be involved in the development of severe malaria [23]. 458 HBV-infected Vietnamese with well-characterised clinical profiles including all forms of hepatic disease and 160 non-infected, healthy Vietnamese individuals were enrolled. The homozygous allele C at the position 17470 in the IFN-α receptor was detected more frequently in HBV-infected patients compared to healthy controls (OR: 2.56; 95% CI = 1.46–4.73, P<0.001). Another polymorphism, leading to an exchange from L to V at codon 168, was found to be protective. Patients being homozygous at this locus were present...