chain of events is that it may take several years for autoimmune mechanisms to overwhelm β-cells. Naturally, the question was raised whether development of clinical IDDM could be predicted by identification of some of the autoimmune markers. The answer is neither simple nor wholly convincing. However, the picture today is far clearer, due to substantial inputs from several elegant research studies, and the study by Tuomilehto *et al.* adds more useful information.

The screening test needed to predict IDDM must have a high specificity since this disease is relatively infrequent in the general population having an overall prevalence of 0.3%, 5% in first degree relatives and 50% in identical twins of IDDM patients. Over the years, several such markers have been proposed but none has been proved to be ideal.

Cytoplasmic islet cell antibodies (ICA) and insulin autoantibodies (IAA) are two widely used immunological tools. The presence of ICA has a positive predictive value of 80%, when IDDM patients were followed for 9 years.[3] Concentration of IAA is believed to reflect the rate of autoimmune damage.[4] The degree of β-cell destruction is indicated by a metabolic marker; diminished first phase insulin secretion.[5] Often, these tests have been combined to enhance their predictive value. Prospective studies, incorporating genetic investigation, first phase insulin secretion and islet cell antibodies have identified non-diabetic individuals whose risk of developing clinical IDDM within 5 years was at least 50%.[6,7]

The presence of antibodies has now been included in this battery of tests. Anti-GAD antibodies may well be the primary immunological marker in IDDM, since they appear early in the disease. At the time of diagnosis of IDDM, about 75% of the patients are positive for anti-GAD.[8,9] A high sensitivity and specificity of anti-GAD estimated in sera stored from earlier pregnancies in patients subsequently developing IDDM is worthy of note. Though simple and well executed, this study could have provided much more information, if it had been performed in a prospective manner. Moreover, as also emphasized in the editorial in the same issue of the journal, a larger number of controls, should have been included to define the specificity further.

What are the practical implications of this study? Identification of a highly sensitive and specific marker early in the life span of high risk individuals, coupled with estimation of other indicators—HLA, other autoantibodies, first phase insulin secretion—may permit the trial of various strategies of primary prevention including the use of free radical scavengers, immunomodulation, oral antigen administration and antigen tolerization. Our eventual aim should be to screen a whole population, perhaps even at birth, for diabetogenic genes and specific autoantibodies. The susceptible individuals might undergo trials of newer strategies (such as cow milk deprivation[10,11]) as a possible method of true primary prevention.

REFERENCES

1 Wolf E, Spencer KM, Cudworth AG. The genetic susceptibility to Type I diabetes: Analysis of the HLA-DR association. *Diabetologia* 1983;**24**:224–30.
2 Todd JA, Bell JI, McDevitt HO. HLA-DQ beta gene contributes to susceptibility and resistance to insulin dependent mellitus. *Nature* 1987;**329**:599–604.
3 Dib S, Vardi P, Connelly J, Eisenbarth GS, Soeldner JS. Absence of transient immunologic or metabolic abnormalities in short term discordant monozygotic twins for IDDM. *BMJ* 1986;**292**:1670.
4 Vardi P, Keller R, Dib S, Eisenbarth GS, Soeldner JS. Log linear correlation of CIAA with age in new onset Type I diabetic. *Diabetes* 1988;**37**:78A.
5 Ganda OP, Srikanta S, Brink SJ, Morris MA, Gleason RE, Soeldner JS, *et al.* Differential sensitivity to beta cell secretagogues in 'early' Type I diabetes mellitus. *Diabetes* 1984;**33**:516–21.
6 Rogbert J-J, Deschamps I, Chevenne D, Roger M, Mogenet A, Boitard C. Relationship between first-phase insulin secretion and age, HLA, islet cell antibody status and development of type I diabetes in 220 juvenile first-degree relatives of diabetic patients. *Diabetes Care* 1991;**14**:718–23.
7 Krischer JP, Schatz D, Riley WJ, Spillar RP, Silverstein JH, Schwartz S, *et al.* Insulin and islet cell autoantibodies as time-dependent covariates in the development of insulin-dependent diabetes: A prospective study in relatives. *J Clin Endocrinol Metab* 1993;**77**:743–9.
8 Atkinson MA, Maclaren NK, Scharp DW, Lacy PE, Riley WJ. 64 000 M autoantibodies as predictors of insulin-dependent diabetes. *Lancet* 1990; **335**:1357–60.
9 Thivolet CH, Tappaz M, Durand A, Petersen J, Stefanutti A, Chatelain P. Glutamic acid decarboxylase (GAD) autoantibodies are additional predictve markers of type I (insulin-dependent) diabetes mellitus in high risk individuals. *Diabetologia* 1992;**35**:570–6.
10 Virtanen SM, Rasenen L, Aro A, Lindstrom J, Sippola H, Lounamaa R, *et al.* Childhood diabetes in Finland study group. Infant feeding in Finnish children 7 years of age with newly diagnosed IDDM. *Diabetes Care* 1991; **14**:415–17.
11 Karjalalainen J, Martin JM, Knip M, Llonen J, Robinson BH, Savilahti E, *et al.* A bovine albumin peptide as a possible trigger of insulin dependent diabetes mellitus. *N Engl J Med* 1992;**327**:302–7.

ANOOP MISRA
Department of Medicine
All India Institute of Medical Sciences
New Delhi

TNF-α alleles and susceptibility to cerebral malaria

McGuire W, Hill AVS, Allsopp CEM, Greenwood BM, Kwjatkowski D. (Department of Paediatrics and Molecular Immunology Group, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.) Variation in the TNF-α promoter region associated with susceptibility to cerebral malaria. *Nature* 1994;**371**:508–11.

SUMMARY

The host response plays an important role in the pathogenesis of infectious disease. One of the mediators of this response is tumour necrosis factor-α (TNF-α) and fatal cerebral malaria is associated with increased levels of this cytokine. There are indications that there is a genetic basis for the variation in TNF-α production. Two polymorphic forms of the TNF-α gene (TNF1 and TNF2) have been identified. The TNF2 allele has been shown to be associated with a higher level of TNF expression.

The authors of this study compared 376 Gambian patients who had cerebral malaria with control groups of children frequency matched for area residence. The control groups were

Group I. Children with mild illnesses other than malaria
Group II. Children with *Plasmodium falciparum* malaria without any complications
Group III. Children with severe malarial anaemia but with no cerebral complications

TNF-α gene polymorphism was typed by amplifying the region by the polymerase chain reaction (PCR) followed by hybridization with allele specific oligonucleotides.

Homozygotes of the TNF2 allele had an increased risk (relative risk 7) for death or severe neurological sequelae due to cerebral malaria. This association was shown to be independent of HLA class I and class II variation. (Even though the TNF-α gene is located close to the HLA locus.) As the TNF2 allele is maintained at a gene frequency of 0.16 in The Gambia, this suggests that the increased risk of cerebral malaria in homozygotes is counterbalanced by some biological advantage.

COMMENT

Cerebral malaria is a complication of infection with *Plasmodium falciparium* (*P. falciparum*) and is more often fatal than *P. vivax* infection. Some survivors are also left with neurological sequelae. Sequestration of parasites within the cerebral blood vessels is a prominent feature of this condition and the cerebral blood vessel bed is characteristically plugged with heavily parasitized erythrocytes. Other features are petechial haemorrhage and oedema.

Parasite–host interactions have nowhere been as clearly illustrated as in malaria. The parasite is obviously important for cerebral malaria to occur. In *P. falciparum* this complication has been shown to be associated with the expression of a protein 'the knob protein, which is important for cytoadherence'. Cerebral malaria has not been shown to occur in *P. vivax* infections. A situation similarly to this exists in CBA mice infected with *P. berghei* but not in those infected with *P. yoelli*.

Host factors too play a role. Many studies are directed towards understanding why only a small proportion of individuals infected with *P. falciparum* get cerebral malaria and one of the factors that mediates this is the pre-existing immune status of the host. Another factor, acting perhaps independently, is the production of the cytokine TNF.

TNF-α is a gene which has been shown to exist in two allelic forms: TNF1 and TNF2. These differ in a region located 308 nucleotides upstream to the transcription start site. Polymorphisms upstream to the transcription start site of a gene have been shown to be associated with variations of gene expression.

The TNF2 allele has been shown to be associated with a higher level of expression of the cytokine than the TNF1 allele. This study sought to determine the influence of the TNF-α alleles in the pathogenesis of cerebral malaria and its outcome. The polymorphism was typed by amplifying the target region by PCR and hybridization with allele specific oligonucleotides. The frequency of TNF2 homozygotes in cerebral malaria was significantly higher than in non-malaria or mild malaria controls. It was even higher in those cases of cerebral malaria who died or developed gross neurological sequelae. It is interesting that in cases of severe malarial anaemia without cerebral complications there was no increase in the frequency of TNF2 homozygotes.

The association of high levels of TNF with cerebral malaria of increasing severity has been demonstrated by Grau *et al.*[1] and Kwiatkowski *et al.*[2] Grau *et al.* measured serum TNF in 65 malarial children (mean age 5.3 years). The 10 patients who died had significantly higher levels of TNF [mean (SE) 709 (312) pg/ml] than the 55 who survived [189 (32) pg/ml]. In patients who died shortly after admission, the TNF levels were shown to be the highest. The association of mortality with higher levels of TNF in this study was also apparent if the patients were divided into three groups on the basis of serum TNF concentration.

Group I. <100 pg/ml (mortality 1/24)
Group II. 100–500 pg/ml (6/37)
Group III. >500 pg/ml (3/7)

However, TNF levels in the cerebrospinal fluid were normal. Kwiatkowski *et al.*,[2] in a larger study showed that plasma TNF is significantly higher in uncomplicated *P. falciparum* malaria than in other illnesses. However, compared to uncomplicated *P.falciparum* malaria, the levels of TNF in cerebral malaria survivors were twice as high. TNF levels in fatal cases were ten times higher than in those with uncomplicated malaria. These studies clearly show that high levels of TNF are not only associated with cerebral malaria, but also with an adverse outcome of the complication.

The TNF2 allele is, presumably, but one of the possible host factors affecting susceptibility to cerebral malaria and influencing the outcome of the disease. TNF-α is produced predominantly by activated macrophages. It is also produced by lymphocytes, NK cells, astrocytes and microglia. Of particular relevance is the ability of TNF to upregulate the expression of adhesion molecules in the cells of the vascular endothelium. These adhesion molecules mediate the binding of parasitised red blood cells to the endothelium. Over production of nitric oxide by TNF may also contribute to disturbances in neurotransmission in cerebral malaria.

TNF-α production is stimulated by helper T cells. The role of helper T cells in the induction of high levels of TNF-α in cerebral malaria has been convincingly demonstrated in a mouse model.[3] However, T cells and macrophages have been shown to be protective in malarial infection. As Grau *et al* write,[3] it is a central theme in immunopathology that cells participating in the immune response may have both beneficial and deleterious effects, depending on their degree of activation, timing and location.

In fact more and more cytokines are being implicated in adverse reactions to infections. Interferons are responsible for the malaise and myalgia of viral fevers and the degree of discomfort correlates with the interferon level. Another mediator of fever is IL-6. IL-1 which is also an inducer of TNF, directly or indirectly contributes to the host discomfort.

The relatively high frequency of the TNF2 allele in The Gambia, in spite of its association with cerebral malaria, indicates that it may have been positively selected in that region during the course of evolution. The authors suggest that heterozygotes may be protected against a broad range of infections. The homozygous allele may also protect from conditions other than cerebral malaria.

The subjects of the studies quoted are from Africa. The situation in India is somewhat different. Malaria trans-

mission, particularly of *P. falciparum* malaria, is less intense and its role as a killer of children not as important. However, the study demonstrates the role of cytokine gene regulation in determining the response of a host to a parasite. It also underlines the dual nature of the immune response and its potential for being a double edged sword.

REFERENCES

1 Grau GE, Taylor TE, Molyneux ME, Wirima JJ, Vassali P, Hommel M, *et al.* Tumour necrosis factor and disease severity in children with falciparum malaria. *N Engl J Med* 1989;**320**:1586–91.

2 Kwiatkowski D, Hill AVS, Sambon I, Twumasi P, Castracane J, Manogue KR, *et al.* TNF concentration in fatal cerebral, nonfatal, cerebral and uncomplicated *Plasmodium falciparum* malaria. *Lancet* 1990;**336**:1201–4.

3 Grau GE, Fajardo LF, Piguet PF, Allel B, Lambert PH, Vassali P. Tumour necrosis factor (cachectin) is an essential mediator in murine cerebral malaria. *Science* 1987;**237**:1210–11.

SUBRATA SINHA
Department of Biochemistry
All India Institute of Medical Sciences
New Delhi
