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Review article

Do genetic factors play a role in the pathogenesis of diabetic microangiopathy?

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In this review we summarize the circumstantial evidence suggesting that genetic factors may play a role in susceptibility to diabetic small vessel diesease.

Clinical studies

The date of appearance and rate of development of small vessel disease, although generally related to duration of disease, shows marked individual variation. It is generally accepted that at least 20% of patients with Type 1 (insulin-dependent) diabetes do not develop these complications even as late as 30–40 years after the onset of the disease [1, 2]. On the other hand, a small minority of patients (perhaps $< 1\%$) may have severe retinopathy after only 5–7 years of disease. It has not been possible to correlate these strikingly different clinical courses with differences in metabolic parameters. Similarly, patients with unrecognized and presumably metabolically mild Type 2 (non-insulin-dependent) diabetes can present to the ophthalmologist with advanced diabetic eye disease [3]. Of course, these patients may have had hyperglycaemia for a long time before seeking medical care.

Studies of the prevalence and severity of retinopathy and nephropathy in forms of secondary diabetes are also of interest. Since one is dealing with non-genetic forms of diabetes, the small vessel disease might be different from that seen in Type 1 or Type 2 diabetes. Pitfalls of this approach include the fact that some of these patients may indeed carry diabetic genotypes and the duration of these diseases tend to be too short to allow for the full development of vascular lesions. The degree of hyperglycaemia may also be quite mild. Nevertheless, it is of interest to note that both diabetic retinopathy, as well as nephropathy, seem generally less common and, especially, less severe (proliferative retinopathy is rare) in secondary than in idiopathic (genetic) diabetes [2].

In addition, there are a few scattered reports in the literature suggesting that occasionally diabetic-like vascular lesions of the eye and kidney appear in normoglycaemic individuals [2]. In a few of these reports, repeated glucose tolerance tests revealed normal carbohydrate tolerance. One might speculate that these rare cases are examples of extremely strong genetic susceptibility to diabetic small vessel disease. The opposite situation, i.e., microangiopathy due only to metabolic factors with negligible genetic susceptibility, may also exist and is perhaps more common.

Many of the studies of microangiopathic complications giving the sex of the patients show that men are considerably more susceptible than women [4]. Whether this sex predilection is genetically related or not is unclear, but it seems worthy of further studies.

A few identical twin studies in the context of microangiopathy have been reported. Pyke's group [5] has suggested that identical twins concordant for Type 1 diabetes seem also to be concordant for retinopathy. Further, they suggested that retinopathy was more frequent and more severe in Type 1 diabetic concordant than discordant twins. HLA associations studied by these authors have shown a higher frequency of the phenotype DR3/DR4 in Type 1 diabetic concordant than discordant twins. This suggests that the HLA-related genetic liability of Type 1 diabetic concordant twins is different and stronger from that seen in those who are discordant [6]. These same authors were less certain of this finding in Type 1 diabetic patients in a subsequent report [7]. Thus, it may be that the higher HLA-related genetic liability in the concordant pairs is associated with more intense complications. This association could be mediated by a more severe diabetic metabolic defect in the DR3/DR4 twins or by a different and unknown mechanism.

The review of epidemiological studies of diabetic complications in various countries is difficult because of the inadequacy of many of the reported studies and/or because of incomplete reports. However, it seems that the prevalence of retinopathy in Type 1 diabetic patients is similar in Japan, India, Europe, and USA [1, 8]. Data on severity of the complications are usually not available. One report on the rarity of diabetic retinopathy in Africa [8] needs confirmation.

Capillary basement membrane width and other biological indicators

Since histological studies in vivo of small vessels in the human eye are impossible and in the kidney are difficult, many studies of the skeletal muscle capillary basement membrane width have been conducted. All, however, have to be qualified by the caveat that we do not know whether the muscle lesion is caused by the same mechanisms involved in the eye and kidney. Indeed, there are probably important differences since the muscle defect always seems to be self-limited unlike the eye and kidney lesions. There is no conclusive evidence that the muscle lesions reflect renal and eye damage accurately.

The earliest studies reported by Siperstein et al. [9] suggested that skeletal muscle capillary basement membrane width was present before the onset of diabetes. These earlier reports were both confirmed [10–12] and refuted [13, 14]. However, more recently, studies of identical Type 1 diabetic discordant twins [15–17] have found that capillary basement membrane width can be absent in some of the diabetic twins (with disease for up to 24 years) and present in some of their non-diabetic twin-mates. Therefore, it seems that at least moderate increments of capillary basement membrane width do not always correlate with the diabetic metabolic defect. Detailed studies of the eyes and kidneys of these twins, as well as IgG and albumin deposition in skeletal muscle (see below), would be of interest.

During genetic studies of Type 1 diabetic families, we have found that non-diabetic siblings of Type 1 diabetic probands, who are HLA identical to the probands, have marked deposition of IgG and albumin in the extracellular membranes of their skeletal muscle [18]. This was not seen in the non-HLA identical siblings [18]. Albumin and IgG depositions are histological indicators of diabetes [19, 20], but we do not know whether they are mediated by hyperglycaemia or not. Since the non-diabetic siblings had normal glucose tolerance and glycosylated haemoglobin, any diabetic disturbance of metabolism, if present at all in these siblings, would have to be very mild and intermittent; alternatively, a different mechanism may have been present. The capillary basement membrane width was the same in the non-diabetic HLA-identical, and non-HLA-identical siblings and was not different from normal subjects [18]. Thus, capillary basement membrane width increment and albumin and IgG deposition may result from different mechanisms. However, Marks et al. [21] have reported increased capillary basement membrane width in the non-diabetic, HLA-DR4-positive parents of Type 1 diabetic patients. Apparently this relationship was not seen in the unaffected siblings of the probands.

The twin studies and the other HLA studies strongly suggest that the diabetic lesions seen in the skeletal muscle are partly determined by genetic factors. Whether this reasoning is also true for the vascular lesions in the eye and kidney is less certain.

Table 1. Main reports in the literature dealing with HLA association with microangiopathy in Type 1 diabetes

Author	Complications		HLA-association
Becker et al.[a] [54]	Retinopathy	133	None[b]
Moller et al. [55]	Retinopathy	99	B15 (proliferative retinopathy)
Cudworth and Bodansky [4]	Retinopathy	65	B15 increased (proliferative retinopathy)
de Moerloose et al. [56]	Retinopathy	39	B8 increased (proliferative retinopathy)
Standl et al. [57]	Retinopathy	46	B8 increased (severe retinopathy)
Bertrams et al. [61]	Retinopathy	50	DR4 increased (proliferative retinopathy)
Jervell and Solheim[c] [58]	Retinopathy (blind)	63	None
Cove et al. [59]	Retinopathy	22	None
Barbosa et al. [32]	Retinopathy	200	B15 increased[d] (proliferative retinopathy)
Bodansky et al.[e] [31]	Retinopathy	133	None
Johnston et al. [34]	Retinopathy	56	None
Dornan et al. [35]	Retinopathy	52	DR4 increased DR2 decreased
Gray et al. [33]	Retinopathy	82	B7 decreased
Danielsen et al. [29]	Retinopathy	42	B15 decreased (proliferative retinopathy)
McCann et al. [30]	Retinopathy	49	C4B2.9 increased[f] (severe retinopathy)
Barbosa et al. [36]	Nephropathy	110	None
Jervell and Solheim [58]	Nephropathy	126	None
Jones [quoted in 4]	Nephropathy	40	B8 increased
Barbosa [38]	Nephropathy	99	None
Christy et al. [60]	Nephropathy	26	DR3/DR4 increased
Saner et al. [38]	Nephropathy	60	None
Walton et al. [62]	Nephropaty	47	None

[a] Type of diabetes and duration of disease not given; [b] A1 and B8 were increased in those without retinopathy; [c] lacked control group; [d] only in patients with onset of disease between 15 and 40 years of age; [e] Type 1 diabetic control group had no retinopathy; [f] rare variant at the locus C4B of complement

Prospective renal biopsy studies of diabetic kidney recipient patients have shown almost always increased glomerular capillary basement membrane width, arteriolar hyalinosis, glomerular IgG and albumin deposition, and mesangial thickening as early as 2–4 years after the renal transplant [22–24]. The rate of progression, however, is extremely variable in these patients. In

some, lesions have progressed to advanced stages of diabetic nephropathy in 8–10 years requiring another kidney transplant. In other patients, the lesions have failed to progress [25]. Retrospectively, no relationships have been found between glycaemia, blood pressure, or duration of disease before nephropathy appeared in the native kidneys, on the one hand, and the striking individual differences in progression of the allograft nephropathy, on the other. The course of events in these patients seems compatible with the existence of genetic factors providing susceptibility (or resistance) to progressive (clinically significant) renal vascular disease.

Genetic markers

One or more genetic factors providing susceptibility to Type 1 diabetes reside at locus/loci in the HLA region [26]. Since there is a reasonable amount of evidence that Type 1 diabetes is, at least in some cases, an autoimmune disorder [27] and the existence of immune response genes in the HLA region is strongly suspected, it has been speculated that one or more of these immune genes are mutant and thereby provide the susceptibility for Type 1 diabetes. On the other hand, there is very little evidence that the microangiopathic complications are autoimmune in nature. Although we have described IgG deposition in extracellular membranes of skeletal muscle [20] and kidney [23] of diabetic patients and some of their non-diabetic siblings [18], and more recently Falk et al. [28] reported evidence for complement activation in the diabetic kidney, all these may be secondary phenomena. An a priori argument against likely involvement of HLA genes in microangiopathy would have been the fact that Type 2 diabetic patients, who show no association with HLA, can develop eye and kidney lesions indistinguishable from those seen in Type 1 diabetic patients. Thus, there was little a priori reason to believe that genes in the HLA region would be involved in modulating the susceptibility of Type 1 diabetic patients to microangiopathy except, perhaps, by resulting in more severe metabolic defects in particular sub-sets of patients. However, many HLA studies have been conducted in the context of diabetic microangiopathy, especially diabetic retinopathy in Type 1 diabetes. Unfortunately, none of these studies met all the desirable criteria (see below) and many were of little value because the number of patients was too small, patient characterization was incomplete or appropriate controls were not used.

We have read 31 publications (many of them Letters to Editors) dealing with this subject. We decided to select 21 of these publications, some of them abstracts, to include in Table 1 because they meet some of the minimum requirements which we think are necessary to obtain interpretable results. Any reports of less than 20 patients are neither included nor discussed because very small studies of HLA frequencies are extremely sensitive to sampling bias.

Fourteen of the studies shown in Table 1 deal with retinopathy, six with nephropathy, and one with both. Five out of the 12 retinopathy studies found an association between B15 or DR4 (alleles in linkage disequilibrium, i.e. often inherited together) and retinopathy, in most cases proliferative retinopathy. One study found a negative association with B7 and two studies found a positive association with B8. One small recent study from Iceland [29] found a negative association between proliferative retinopathy and B15 (that is B15 was less common among patients with proliferative disease). This is the only study to find this type of relationship. McCann et al. [30] were the only ones who typed components of the complement system in patients with retinopathy. The rare variant, C4B2.9, seemed more frequent among those with proliferative retinopathy. The other complement component studied in this context, i.e. Bf, did not seem associated with retinopathy [29]. More studies of complement are necessary. Five out of the 12 retinopathy reports found no HLA associations (in most cases, studies of proliferative retinopathy). The main limitations of these studies have been adequately reviewed [4]. However, the possible importance of stratification by age at diagnosis has often been neglected with one exception [31]. In our report of a large group of patients with proliferative retinopathy [32], the B15 association was only present for those patients with onset of disease after the age 15 years. In two of the most recent reports [33, 34], no association was found between B15 and proliferative retinopathy. One of these reports [34] and a previous negative study by Bodansky et al. [31] used as control subjects diabetic patients without detectable retinopathy (the best comparison group) and found no B15 association. The latter study [31] included stratification for age at diagnosis, with the cut-off at age 10 instead of 15 years as used by us [32].

An additional recent report [35] attempted to analyze microangiopathy in function of both metabolic control and HLA types. This would be, of course, the best approach had metabolic control been analyzed prospectively. Retrospective analysis of metabolic control, especially in the absence of glycosylated haemoglobin, may be misleading. However, these authors found that the frequency of DR4 (in linkage disequilibrium with B15) was lowest in patients with poor metabolic control and little or no retinopathy and was highest in patients with retinopathy and best metabolic control. The results were thought to be compatible with an HLA-related modulation of vascular susceptibility to the disturbed metabolic milieu. This is an attractive concept, indeed. More and larger studies of this nature are necessary, but should be carried out prospectively.

For nephropathy the evidence for an association with HLA is practically nil. Only two small studies out of six suggested an association with B8 and DR3/DR4. Our earlier report on HLA and nephropathy [36] has been repeatedly quoted as suggestive of an association between B8 and nephropathy, but this is not the case; we simply showed the now well-recognized association

between Type 1 diabetes and B8. HLA-B15 was not increased in this study (it has been increased in all our subsequent studies of Type 1 diabetic patients) because in 1974 the typing for B15 was probably not entirely reliable. In additional studies of advanced diabetic nephropathy, we found no evidence for HLA association [37, 38].

There is clinical parallelism between eye and renal diabetic microangiopathy. This is particularly evident in Type 1 diabetic patients with advanced diabetic nephropathy. In more than 800 of these patients seeking kidney transplantation at the University of Minnesota less than 1% lacked retinal lesions and in many cases they had proliferative retinopathy (unpublished observations). On the other hand, many patients with proliferative retinopathy seem to have relatively little evidence of diabetic nephropathy, as assessed by Albustix-positive proteinuria. Most of the patients with proliferative retinopathy we studied [32], who showed an association with B15 (when age of onset was between 15 and 40 years), had been recruited from the Diabetes Retinopathy Study. In this study, patients with abnormal serum creatinine were excluded. Therefore, this patient sample was enriched with patients who had discrepant retinal versus renal microangiopathy. Since we did not find the same B15 association for the patients with nephropathy, we speculated that there may be two types of proliferative retinopathy insofar as the genetic susceptibility to retinal microangiopathy is concerned [37]. These would include the retinopathy seen in association with advanced renal disease not associated with HLA genes and the retinopathy usually progressive in the absence of clinically significant nephropathy associated with B15 for individuals with age at diagnosis after age 15 years [37]. However, additional population studies and search for multiplex families with retinopathy only or retinopathy and nephropathy are necessary to test this hypothesis. It seems wise, however, to be aware of the possibly important pathogenic differences between diabetic retinopathy and nephropathy. More studies should be conducted to elucidate this apparent dichotomy.

Assuming that some of the associations discussed above and shown in Table 1 are biologically meaningful, and reflect HLA-related genetic susceptibility to vascular disease, one may ask: what is the mechanism by which a gene in the HLA region may provide susceptibility for retinopathy? One possibility is that certain HLA types are associated with more severe metabolic defects, thereby resulting in more widespread or intense vascular damage. Other possible links would be insulin antibodies or immune complexes. HLA-B15 and -DR4 have been found to be associated with higher titres of insulin antibodies [31, 39]. However, the evidence that insulin antibodies mediate vascular damage is most tenuous and microangiopathy can be seen in patients who have never received insulin [4, 31]. Several reports have described associations between immune complexes and severity of retinopathy [31, 40, 41]. The fact that no relationship has been found between HLA and immune complexes mitigates against their role as mediators of any possible HLA gene effect on microangiopathy, although it does not rule out this possibility completely.

In conclusion, it is still possible that a sub-set of Type 1 diabetic patients with certain HLA types are more susceptible to complications, especially proliferative retinopathy, although this HLA-related effect is probably weak. This, of course, does not negate the existence of important non-HLA-related genetic factors involved in susceptibility to diabetic small vessel disease.

Cell mediated immunity

Although there is considerable evidence that Type 1 diabetes is caused, at least in some cases, by dysregulation of the immune system with prominent cell mediated abnormalities, very few studies of this nature have been conducted in the context of microangiopathy. In one report [42] it was claimed that uveoretinal antigen produced significant inhibition of leucocyte migration using lymphocytes from patients with proliferative retinopathy. The specificity of this response is, however, questionable [43].

Other studies

Acetylator polymorphism

A relationship between the fast acetylator phenotype and Type 1 diabetes [44] and a predominance of this phenotype in patients with neuropathy [45] have been claimed. However, these findings have been challenged [46] and the role of this genetic marker in diabetes is uncertain at the present time.

Restriction fragment length polymorphisms

DNA insertions in the 5′ flanking region of the insulin gene (in chromosome 11) have been found associated with Type 2 diabetes [47, 48]. In some studies, associations with hyperlipidaemia [49] or atherosclerosis [50] have also been suggested. However, no relationships have been claimed with microangiopathy.

Future studies

As suggested by Cudworth and Bodansky [4], any future studies of diabetic microangiopathy with genetic markers should include a group of diabetic control patients without complications. Studies of this nature would ideally meet the following criteria: (a) ethnically homogeneous Type 1 diabetic patients with long duration of

disease (preferably 30 years or longer) with proliferative retinopathy and/or nephropathy and patients with documented absence of these complications. Ideally, studies of retinopathy would include stereophotos and nephropathy quantitative studies of microalbuminuria. The two groups would be matched at least for duration of disease, sex, and blood pressure. (b) The absence or presence of eye and kidney lesions should be documented in adequate detail. (c) The patients should be analyzed both as a group and after stratification by age of diagnosis, sex, and other variables to search for associations which may be present only in sub-sets of patients. (d) Besides studying the HLA, especially B and DR antigens, other genetic polymorphisms, which in some cases have been claimed to be associated with Type 1 diabetes, could also be tested. These might include Kidd [51], Lewis, ABO [4, 52], complement proteins [53], etc. The status of the chlorpropamide-induced alcohol flush as a genetic marker is uncertain, but might also be studied since it has been claimed to be more frequent in Type 2 diabetic patients without complications [4].

Whenever possible, studies of genetic polymorphisms should be carried out in patients whose past metabolic control is documented, as performed by Dornan et al. [35]. This would permit the study of possible genetic effects in patients with different levels of control.

More studies of identical and dizygotic twins, as well as studies of affected non-twin sibling pairs for concordance of microangiopathy also need to be conducted. Preferably these studies should be carried out in conjunction with typing for genetic markers and immunological studies, such as immune complexes and possibly insulin antibodies.

Studies of animal models of spontaneous (genetic) insulin-dependent-like disease, such as the BB rat, the non-obese diabetic mouse, and the Chinese hamster; prevalence and rate of progression of microangiopathic-like lesions would also be of interest.

Conclusions

There is no direct and conclusive evidence that genetic factors play a role in the pathogenesis of microangiopathy in diabetes. Associations between HLA antigens and complications, which would contribute conclusive evidence of a genetic contribution to the genesis of these complications, have been reported but not consistently confirmed. On the other hand, circumstantial evidence from studies of the natural history of the disease and its complications and from twin studies strongly suggest that genes do contribute to the susceptibility to small vessel disease. The most important genes providing this susceptibility are probably unrelated to HLA. Even if the total genetic contribution to susceptibility for microangiopathy turns out to be small in comparison with that provided by metabolic factors, their knowledge early in the course of the disease would serve the important role of identifying subjects in need of more intensive diabetic management to prevent vascular complications.

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