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The role of TNF and its receptors in Alzheimer's disease

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Abstract

Tumor necrosis factor (TNF) is an important proinflammatory cytokine that is upregulated in Alzheimer disease (AD) patients and involved with AD genes. Several TNF promoter polymorphisms that increase expression are associated with inflammatory and infectious diseases. We previously reported results that detected a AD associated region near the TNF gene. Using family-based association tests we also reported an association between AD and a TNF haplotype in sibling-pair families, and a significant increase in the mean age of onset for a group of African-American AD patients carrying this same haplotype. Previous reports have shown that that the chromosome 1p and chromosome 12p regions are linked to late-onset AD. These two regions harbor TNF receptors (TNFR) 2 and 1, respectively, and binding to them mediates biological effects of TNF. We found a significant asssociation of a TNFR2 exon 6 polymorphism with late-onset AD in families with no individuals possessing the APOE E4E4 genotype under a dominant model. We found no significant association of three polymorphisms in the TNFR1 gene to AD. These results provide further evidence for the involvement of TNF in the pathogenesis of AD.

Keywords: TNF; TNFR1; TNFR2; Free radicals; Polymorphisms; Inflammation; Alzheimer Disease

1. Introduction

1.1. Tumor necrosis factor

Tumor necrosis factor (TNF) is one of the main proinflammatory cytokines that plays a central role in initiating and regulating the cytokine cascade during an inflammatory response [53,89]. Expression of TNF can be induced by bacterial lipopolysaccharide (LPS), mitogens and viruses [149]. It participates in local and systemic events involving inflammation. Along with interferon gamma (IFN-γ), TNF is a potent paracrine stimulator of other inflammatory cytokines, including interleukin-1 (IL-1)-1), IL-6, IL-8, and granulocyte-monocyte colony-stimulating factor. They in turn continue and amplify the response in various ways, such as activating T and B cells and stimulating acute-phase protein synthesis, including colony-stimulating factor [26, 124].

TNF's effect on vascular endothelial cells include, morphological changes, modulation of expression of surface antigens, and elaboration of procoagulant activity [see 10 for review]. TNF increases the expression of adhesion molecules on the vascular endothelium which can allow leukocytes and immune cells, such as neutrophils and macrophages, to be attracted to areas of tissue damage and infection [6,48]. For example, neutrophils migrate into the intravascular space and release biologically active substances like lysozyme and hydrogen peroxide which leads to degranulation [11]. LPS activated mononuclear phagocytes are the major producer of TNF and TNF-activated phagocytes engulf and clear infectious agents and cellular debris [55,149]. TNF is the most abundant product of activated macrophages [11]. In the presence of IFN-γ, the synthesis of TNF by LPS activated macrophages is further enhanced causing them to differentiate and activate nitric oxide synthase which, in turn induces nitric oxide production that effects the killing of microorganisms. In the absence of IFN-γ, TNF stimulates macrophages to differentiate along a pathway that results in the production of insulin-like growth factor-I [161]. Higher levels of TNF are generally related to the severity of the response, but whether greater TNF production causes more severe inflammation or whether more

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severe inflammation elicits increased TNF synthesis is unclear [55].

However, TNF is also important in limiting and terminating inflammation, enhancement of the repair of the damage, and angiogenesis. This is illustrated in TNF knockout mice. When homozygous TNF gene knockout mice were infected with the bacterium, *Corynebacterium parvum*, there was little or no initial response but the mice went on to develop a severe and fatal inflammatory reaction [91]. Heterozygous mice for the TNF gene were more susceptible to endotoxin-induced shock and to certain infections while normal mice developed an inflammatory response that resolved. Thus, TNF appears to have a dual role of being pro-inflammatory in the early phase of a response to an infection and an anti-inflammatory function in the later phases of the response in order to limit the extent or duration of the inflammation and to promote repair [89,91].

Expression of TNF mRNA appears to be present at a low levels or absent in the normal brain [see 152 for review]. In the normal rat brain it has been detected in the hypothalmus, hippocampus, cortex, cerebellum and brainstem, however most studies using in situ hybridization methods could not detect transcripts in the brains of mice or rats. The protein also appears to be localized to the same brain regions and at low or undetectable levels. In humans, TNF mRNA has been detected in the basal ganglia, cortex, and deep and sub-cortical white matter regions [156].

Because of its' low levels of expression it has been difficult to determine what its' precise role is in the normal brain. In vitro studies indicate it can suppress activity of glucose-sensitive neurons of the hypothalmus, alter presynaptic a2-adrenergic receptor responsiveness in the median eminence, and modify ion channel permeability in rat hippocampal neurons [152]. TNF is produced by neurons, microglia, and astrocytes, although the latter two may be in response to pathological stimuli [20]. In an inflammatory or diseased state, TNF along with a variety of pro-inflammatory mediators and neurotoxic substances are produced by activated microglia [107]. TNF, IL-1, and IL-6 are the primary cytokine mediators of inflammation that are produced by these cells and they tend to induce each other [124]. TNF also activates NF-κB [98,109] which stimulates the production of many substances, including survival factors such as manganese superoxide dismutase (MnSOD) [107] and the transcription of other cytokines [151], including TNF itself [144,145]. In astrocytes, TNF, along with other substances, is a strong inducer of IL-6 [148].

Compared to other cytokine genes, TNF is highly polymorphic; this may be partly due to its' location between the highly variable class II and class I regions of the MHC or it could also reflect environmental selection due to its importance in many biologic processes [55], including the critical role in inflammation that is described above. The TNF gene is located in the class III region of the MHC at chromosome 6p21.32, is 2,676 bp long and contains 4 exons and 3 introns [70,89]. TNF is produced as a membrane-bound 26 kDa precursor molecule and is cleaved by the enzyme TNF-α converting enzyme (TACE) to produce a soluble 17 kDa active form of TNF [12,103]. Two polymorphisms are located in intron 1 and one polymorphism is located in intron 2, however a total of 8 single nucleotide polymorphisms (SNPs) are located in ~1 kb of the 5′ UTR and promoter region [70].

Some of the promoter SNPs appear to affect transcriptional activation. Reporter gene constructs containing the A allele of the –308 SNP appear to have higher transcriptional activity than with the –308G allele [19,82,157,163] and some in vitro studies have demonstrated higher levels of TNF are released from cells with the –308A allele of TNF [17,85]. Other groups do not report any differences in activation between the two alleles [21,60,69,136]. These conflicting results appear to be the result of the use of different transcription factors and cell lines; in addition various protocols and reporter constructs were used. One study reported the TNF –308 polymorphism affected TNF transcription in both a cell-type and stimulus-specific manner [81]. Although there is binding of a transcription complex to this region in all cell types, it is not the only factor governing whether the promoter region containing the –308A allele results in elevated expression in a given cell type since additional cell-type specific nuclear factors binding to this area are likely to be involved in expression.

One study has found an association between the G allele of the TNF –238 polymorphism and higher TNF production [69], but this was not demonstrated by a previous study [119]. Methodological differences, such as isolated cells vs. whole blood cultures, the amount of endotoxin used to stimulate the cells and differences in study size, could explain these conflicting results. Another explanation could be the presence of a third TNF promoter SNP located at –376. One group reported the –376 SNP was aligned with the binding site for the transcription factor OCT-1 and the –376A allele was found to bind the OCT-1 protein while the G allele did not [78]. The authors reported that the regions located at –238 and –308 did not bind any protein. Since all three polymorphisms are in linkage disequilibrium, the –238 and –308 SNPs effect on expression of TNF may be due solely to the functional –376 polymorphism or a functional site elsewhere.

There is a cluster of five highly polymorphic microsatellites (TNFa-e) surrounding the TNF gene [73,106,146] that have also been associated with altered TNF production. One In vitro study reported the TNFa2 and c2 alleles were associated with higher TNF production while the TNFa6 and TNFc1 alleles were associated with lower TNF production [120]. However, another study found the a2 and a6 alleles were associated with lower TNF synthesis than the a4 and a11 alleles [37]. Different methodologies using different agents for stimulation may explain these results. The TNFa2, b1, c1 alleles seem to be part of an HLA-DR4 extended haplotype DRB1*0401 with HLA-B62 that appears to be associated with high TNF production while another HLA-DR4 extended haplotype, DRB*0401, TNFa6,

b5, c1, HLA-B44 is usually associated with low TNF production. This relationship between the microsatellites and TNF production is probably due to linkage disequilibrium [55].

If the promoter polymorphisms and the microsatellites lead to or are associated with increased TNF expression levels, they could contribute to the course and/or severity of diseases, especially those involving inflammation. Inflammatory conditions that have been reported to be have a positive association with the –308A allele include coeliac disease [35,97], primary sclerosing cholangitis [10], and primary biliary cirrhosis [55]. Interestingly, the –308A allele seems to be protective against ulcerative colitis [17] while the –308G allele is a risk factor [55]. Additionally, the TNF a2, b1, c2, d4, and e1 microsatellite alleles have been found to be positively associated with ulcerative colitis [118], although HLA haplotypes were not included.

A variety of infectious diseases of different etiologies have been associated with the –308A allele. They include, mucocutaneous leishmaniasis [24], trachoma [32], meningococcal meningitis [105], leprosy [127], septic shock resulting from bacterial infections [100], brucellosis [23], and malaria [96,153]. The A allele of the -376 SNP has also shown to be a risk factor for malaria, independent of the –308A allele [78]. Altered TNF expression such as increased or long-term exposure could cause more damage and interfere with the appropriate response to an infectious agent just as a reduction in TNF production could compromise a full immune response. In the case of malaria, the picture is further complicated by the fact that the aforementioned adhesion molecules on the vascular endothelium are also receptors for *Plasmodium falciparum* so that an increase in their expression, due to TNF activation, could preferentially select for the parasite-binding phenotypes that cause malaria [78].

Possible involvement of the SNP at –238 in Hepatitis B and C has been reported [64,65]. Chronically affected individuals were more likely to have the –238A allele than those who had eliminated the virus. The TNF genotype could influence the initial response to viral infection, such as viral neutralization and clearance, or it might affect the long-term outcome of infection [55].

Although the –308A allele has been reported to be associated to several autoimmune diseases or conditions [34,36, 46,63,120,125,138,158–160], most of these associations appear to be due to linkage disequilibrium with HLA haplotypes. The same is true for the microsatellite associations with autoimmune diseases in that they seem to occur as part of extended MHC haplotypes [55–58,102,104,120, 140] although the TNFa2 and c1 associations may be independent of HLA genes [56,104].

2. TNF and Alzheimer disease

The above introduction lays the background as to how TNF seems to play a role in various inflammatory and infectious diseases. We will present results from our laboratory that provide genetic evidence implicating a polymorphic haplotype of TNF in late onset Alzheimer disease (AD). Initially, we reported that a chromosome 6 genome screen detected a putative AD associated region near the TNF gene at chromosome 6p21.3 [28,30,52], which has been confirmed in other genomic scans [49,113]. The TNF –308 and TNF –238 promoter region polymorphisms [150] and the microsatellite polymorphism TNFa [89,92], located approximately seven kilobases upstream of TNF, were genotyped by our group in 145 late onset Caucasian families [28]. The TNF haplotype 2(A)-1(G)-2(99 bp), respectively, was significantly associated (p-value = 0.005) with AD using the SDT program [66]. Two of the TNF alleles comprising this AD haplotype, TNF –308 2 and TNFa 2, have been shown in some studies to be associated with increased TNF production (see previous section). This could lead to the chronic inflammatory state and free radical damage hypothesized to be involved in AD pathogenesis [94,162].

In a case-control study, which included 111 African-American's with Alzheimer disease [114], we did not find any association of the TNF –308 2 allele with AD. We did show a significant increase in age of onset for patients carrying the TNF –308 2 allele (mean age = 73.9) when compared to patients with no 2 allele (mean age = 70.6, p-value = 0.02). We also showed a significant increase in age of onset for individuals with the TNFa 2 allele (99 basepairs) (mean age = 74.4) when compared to all others (mean age = 70.1, p-value = 0.002). The haplotype containing these two alleles along with the –238G allele [2–1-2], as described above was not significantly associated with AD in this study of African-American patients. However, there was a significant increase in mean age of onset for patients carrying the 2–1-2 haplotype (mean age = 73.8) when compared to all others (mean age = 70.7, p-value = 0.04).

Our results of the study of AD in African-Americans [114] and our previous results in primarily Caucasian AD patients [28] may reflect different modifying effects by the TNF gene in different ethnic groups depending on its' interaction with different succeptibility genes and/or risk factors. Also, the family-based association test that was used in the analyses of the families in the candidate gene set identifies susceptibility alleles through identity by descent (IBD) whereas, the analysis of the African-American case-control set is based upon allele identification through identity by state (IBS) in a population. Thus, asssociations in the candidate gene set can be attributed to other genes located nearby that are in linkage disequilibrium with TNF. Some studies have reported an association of the A2 allele of HLA-A gene with an earlier age of onset in sporadic, early onset, and late onset Caucasian AD patients [5,31,112), however, we did not find any association of the HLA-A2 allele to AD nor any lowering of age of onset by A2 in AD patients (primarily Caucasian) in our candidate gene set [28] or in the African-American AD patients [114]. These ob-

servations either indicate some modifying role for TNF in the onset of AD or linkage disequilibrium with another locus nearby.

In a recent study of another TNF promoter polymorphism, C-850T, the T allele was found to increase the risk to AD patients when in the presence of an APOE e4 allele. The odds ratio was 4.6 (95% CI 2.4–9.0) with an APOE e4 allele and those with an APOE e4 allele but no T allele had an odds ratio of 2.7 (95% CI 1.7–4.4) [93]. These results indicate that TNF expression may augment the effects of ApoE.

3. TNF receptors

The biologic effects of TNF are mediated by binding to its' two main receptors, the p55 TNF receptor (TNFR1) and the p75 TNF receptor (TNFR2). The TNFR1 gene is localized to chromosome 12p13.31, contains 10 exons, and codes for a 55/60 kDa membrane receptor [44,70,84]. The TNFR2 gene is located at chromosome 1p36.22, contains 10 exons, and codes for a 75/80 kDa membrane receptor [70, 128,129,134]. Both receptors belong to a superfamily of transmembrane receptors that are defined by a similar cysteine-rich extracellular domain, however, the intracellular regions of TNFR1 and TNFR2 appear to be unrelated [9,90]. Both TNFR1 and TNFR2 are shed from the cell surface to soluble forms [61,79]. In fact, TNFR2 is cleaved by the same enzyme, TACE, that cleaves TNF [55]. These soluble receptors can neutralize the activity of TNF by competing with the membrane-bound forms, however they also stabilize TNF and prevent its degradation [1].

TNFR1 is constitutively expressed at low levels on all nucleated cells, including neurons throughout the brain, and appears to be involved in most TNF-mediated effects in many different cell types [9,25,76,128]. It also signals for fibroblast growth, endothelial cell activation/adhesion, and anti-viral activity, and is the primary mediator of TNF-induced cytotoxicity and apoptosis in non-lymphoid cells [88,142]. Apoptosis and NF-κB activation is linked to an 80 amino acid stretch in the carboxy-intracellular region known as the "death domain" [67].

TNFR2 has a higher affinity for TNF than TNFR1 and binds TNF better at lower concentrations [55]. Although TNFR2 is expressed primarily on cells of hematopoetic origin, it is also expressed on many cell types including neurons throughout the brain, [25,76,128]. It is responsible for signaling pro-inflammatory responses including thymocyte and peripheral T-cell proliferation and apoptosis and participates in B-cell activation [9,45,142,143]. It also can enhance TNFR1-mediated apoptosis [155].

Transgenic and knockout TNF receptor mice have provided further evidence of neurological and inflammatory roles for TNF and these TNF receptors. Overexpression of TNFR2 in transgenic mice resulted in a severe peri-vascular inflammation involving the pancreas, liver, kidney, and lung, accompanied by constitutively increased NF-κB activity in the peripheral blood mononuclear cells [38].

In TNFR2 deficient mice, there was an increase in pulmonary infiltrate after being exposed to heat-killed antigen and increased induction of serum TNF by stimulation of LPS, which suggested to the authors that TNFR2 may have a regulatory role in suppressing inflammatory responses mediated by TNFR1 in certain circumstances [115]. One group knocked-out TNFR2 and TNFR1 in mice and double, knockout mice were generated from crosses of the singly deficient mice [22]. The TNFR-deficient mice showed no overt phenotype, but damage to the neurons caused by a focal cerebral ischemia and epileptic seizures were exacerbated, indicating that TNF serves a neuroprotective function. Also, oxidative stress was increased and levels of the antioxidant enzyme, manganese superoxide dismutase (Mn-SOD), were reduced in the brain cells, indicating that TNF protects neurons by stimulating antioxidant pathways. Injury-induced microglial activation was suppressed in these mice, demonstrating a key role for TNF in injury-induced immune response. In another study by this group, levels of MnSOD were also reduced along with delayed responses of NF-κB activation in TNFR-KO mice after traumatic brain injury compared with that of wild types expressing TNF receptors [137]. A third study found neuronal damage after focal cerebral ischemia-reperfusion was significantly increased in TNFR1 knockout mice when compared to wild type and TNFR2 deficient mice [50]. Also, the mice lacking TNFR1 demonstrated increased degeneration of hippocampal neurons after administration of the excitotoxin kainic acid compared with wild-type and TNFR2 deficient mice. These results suggest TNF plays a neuroprotective role after acute brain insults.

4. TNF receptors and Alzheimer's disease

One study reported that patients with dementia of Alzheimer type [DAT] were found to have more of both types of receptors on T lymphocytes than controls [16]. This could indicate a systemic immune activation in DAT patients as compared with healthy controls. A separate study has investigated a polymorphism in another receptor in this superfamily of receptors, TNFR6 which encodes FAS, a cell-surface receptor involved in apoptosis initiation [42]. They found the promoter SNP in this gene along with APOE4 was associated with early onset Alzheimer disease in a group of Scottish patients. This could indicate the AD risk contributed by APOE4 could be related to a pathway involving apoptosis and this receptor family.

The chromosome area on 12p containing TNFR1 has been linked to late-onset AD in a genomic screen of 54 families [113], and this finding has been followed up and supported by other studies [75,123]. Only a few mutations in TNFR1 from a small number of families have been reported and they occur in the extracellular domain. The

clinical symptoms include fever and severe localized inflammation [47]. There have been three SNPs identified in the gene. One is located in exon 1 at codon 12 (36A 224 G) that does not result in a coding change [117] and two additional SNPs are located in introns 5 and 7 [7]. A highly polymorphic dinucleotide repeat has also been observed in TNFR1 [40].

To investigate whether or not the TNFR1 gene is associated with late onset AD, we used family-based association testing [83] to look at the exon 1 polymorphism in 150 families with at least two affected and one unaffected siblings. There was no significant association of this polymorphism to AD under an additive model (Z score = 2.28, p-value = 0.022). In order to increase our informativeness, we also typed the two other SNPs in intron 5 (C→T) and intron 7 (G→A) [7] but found no significant associations.

We have also previously reported the chromosome 1p region containing the TNFR2 gene as also being linked to AD in set of 145 families with at least two siblings with an age of onset of over seventy years; these families were part of the total National Institute of Mental Health (NIMH) sibling dataset of 266 sib-pair families [29]. We have confirmed the suggestion of linkage to the same 1p terminal region in an expanded set of the 320 late onset families. Using GENEHUNTER we obtained Z scores of 1.98 (p-value = 0.024) to 1.99 (p-value = 0.023) between 18 and 21 cM from the p terminal end of chromosome 1. SIBPAL analyses for marker D1S1597 located at 30 cM from the p terminal end gave a mean IBD of 0.52 (p-value = 0.0329). We obtained even more significant findings for a subset of 125 families that were homozygous for APOE4. GENEHUNTER scores were 3.29 (p-value $<$ 0.001) to 1.99 (p-value = 0.023) covering a distance of 21 cM from the p terminal end of chromosome 1. These were supported by SIBPAL analyses: D1S2845 (mean IBD = 0.58, p-value $<$ 0.001), D1S1612 (0.56, 0.005), and D1S1597 (0.56, $<$ 0.001) located at 9, 16, and 30 cM respectively.

As in TNFR1, we wanted to follow up these linkage results with family-based association testing. In addition to microsatellites located in TNFR2 [9,128], many polymorphisms have been identified in the gene, but some of these are rare [70,110,128]. Exon 6 codes a portion of the transmembrane region and is a cleavage site for soluble TNFR2. A polymorphism located in exon 6 (676 T 224 G) leads to a 196 M 224 R substitution. This polymorphism has been associated with SLE in Japanese populations [80], but is not associated with SLE in Caucasian (United Kingdom) or Spanish populations [2].

We typed this polymorphism in the same candidate gene set as TNFR1. The TNFR2 exon 6 T 224 G polymorphism did not have significantly different genotype frequencies when compared to affected and unaffected siblings. However, we found a significant association of this polymorphism with AD in families with no individuals possessing the APOE E4E4 genotype under a dominant model using family-based association testing (Z score = −2.21, p-value = 0.027) [81]. Mean ages of onset were not significantly different when comparing patients with different genotypes.

5. Discussion

The role of TNF and inflammation in AD has been much discussed and debated [see 106 for review]. There is a lack of immune cell mediation in the brain and there are none of the classic features of inflammation such as edema, swelling, and vascular proliferation. However, the upregulation of many inflammatory mediators including the complement proteins, the primary proinflammatory cytokines mentioned above, and other acute phase proteins have been demonstrated in the AD brain. These mediators co-localize to AD affected areas of the brain with particularly high expression near the pathological features that are found in the AD brain, neuritic plaques and neurofibrillary tangles (NFTs); however they are absent or minimal in unaffected regions of the AD brain. These inflammatory mediators could cause neuronal damage by overstimulating the immune system [71,95,141], which is supported by the fact that induced brain inflammation in rats causes neurodegeneration and memory loss [59]. In fact, inflammation may be one of the final common pathway through which neuritic plaques and NFTs manifest their neurodegenerative defects. This is the conclusion reached by the authors of one study who compared the brains of patients with no history of dementia, but demonstrating a neuropathology indicative of an AD diagnosis to AD patients and non-demented controls and found inflammatory markers and changes were the best predictor of synaptic changes [86].

The three genes demonstrating autosomal dominant transmission which have been found to cause AD in primarily early-onset families may also be involved in this inflammatory model, since amyloid precursor protein (APP), presenilin 1, and presenilin 2, are proposed to cause AD by increasing the production of amyloid β (Aβ) 42 [27,130,132]. Aβ upregulates and activates astrocytes and microglia [107]; these could act like inflammatory cells since they release a myriad of proinflammatory cytokines, including TNF, IL-1, and IL-6 [43,54,77]. In addition, microglia produce cytotoxic and neurotoxic free oxygen radicals [131]. Aβ42 is oxidized in the presence of free radicals [39,165] and aggregates [72] to form neurotoxic AD neuritic plaques [164]. This leads to overstimulation of the immune system [8,74]. Aβ also increases the production of nitric oxide (NO) in the presense of cytokines, which can also lead to neuronal damage [126,133].

The other AD gene, apolipoprotein E (APOE) ϵ4 allele, especially in its homozygous form, is a major risk factor for late-onset AD [41,135,139] and may act by lowering the age at onset of AD [14,33,99]. The APO ϵ4 allele has also been shown to have the least antioxidant activity of the three common alleles [101]. APOE is synthesized and secreted by microglia and astrocytes in the CNS [18]. APOE contains

NF-κB-like consensus sequences, therefore the activation of NF-κB through microglial upregulation by Aβ, cytokines such as TNF, or neuronal injury would increase the expression and release of APOE [3]. Since APOE has been shown to be important for neuritic plaque formation in transgenic mice [4], upregulation of APOE expression could accelerate Aβ deposition and the formation of neurotoxic neuritic plaques resulting in further overstimulation of the immune system [8,74].

TNF increases the production of Aβ and inhibits the secretion of neuroprotective, soluble amyloid precursor proteins (sAPPs) [15]. TNF, which is released by microglia [62,77], activates NF-κB, [98,109] which in turn stimulates the transciption of more TNF [144,145], other cytokines [151], and APOE and its release [3]. This upregulation of both Aβ and APOE by TNF would then lead to increased neuritic plaque formation. TNF has also been shown to upregulate cyclo-oxygenase 2 (COX2) expression [51], which would increase the level of free radicals [116]. COX2 expression has been found to be higher in Alzheimer patients [111], especially within neurofibrillary tangles [108].

A deletion in the α-2-macroglobulin (A2M) gene has been implicated in late-onset AD [13] and it may also be involved with TNF. A2M is an acute phase protein and AD plaque component [122,147] that binds to Aβ [68] and degrades it [121]. Additionally, A2M binds TNF [154] and A2M may be regulated by the release of NF-κB [3], TNF, and other cytokines [87]. Thus, one hypothesis is the deletion in A2M could potentially affect Aβ and TNF binding sites, leading to less degradation, additional plaque formation, and immune overstimulation. However, functional studies of the A2M deletion and its effect upon binding Aβ and TNF would be needed to confirm this hypothesis.

6. Conclusion

TNF plays a pivotal role in the general inflammatory response throughout the body. It is not only involved in the activation of other inflammatory cytokines and the surrounding events during the initial immune response, it is also important in limiting and terminating inflammation to prevent further tissue damage. It is not clear what its role is in the normal brain, but it is clearly upregulated when damage to neuronal cells has occurred. In AD it is also upregulated in and involved with molecules associated with AD, including Aβ, ApoE, and A2M. There is a growing amount of evidence that inflammation is involved in the pathogenesis of AD and TNF may play a role in this process. Several polymorphisms in the promoter of the TNF gene have been found to increase TNF expression and have been associated with various inflammatory and infectious diseases.

We previously reported results of linkage studies that detected a putative AD associated region near the TNF gene at chromosome 6p21.3. We also reported an association using family-based association testing between a haplotype of two TNF promoter polymorphisms and the microsatellite TNFa and AD in the NIMH sibling data set. Furthermore, we found a significant increase in mean age of onset for a group of African-American AD patients carrying this haplotype.

The chromosome 1p terminal region was found to be associated with AD in the same NIMH sibling data set. Other linkage studies have indicated the chromosome 12p region is linked to late-onset AD. These two regions harbor TNF receptors which mediate the biologic effects of TNF. In this paper, we confirmed previous linkage results for this chromosome 1p region in an expanded AD sibling data set. Using family-based association testing, we also found a significant association between a TNFR2 exon 6 polymorphism and late-onset AD and in families with no individuals possessing the APOE E4E4 genotype under a dominant model. We did not find an association for three polymorphisms located in the TNFR1 gene to AD. This involvement and association with TNF, TNFR1, and TNFR2 in late-onset AD provides further evidence for the involvement of TNF and inflammation in the pathogenesis of AD.

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