0031-3998/01/4905-0686
PEDIATRIC RESEARCH
Vol. 49, No. 5, 2001
Printed in U.S.A.


# Increased Frequency of Alleles Associated with Elevated Tumor Necrosis Factor-α Levels in Children with Kawasaki Disease

MICHAEL W. QUASNEY, DAVID E. BRONSTEIN, RITA M. CANTOR, QING ZHANG, COURTNEY STROUPE, HIROKO SHIKE, JOHN F. BASTIAN, TOMOYO MATSUBARA, MOTOKI FUJIWARA, KATSUMI AKIMOTO, JANE W. NEWBURGER, AND JANE C. BURNS

*Division of Critical Care, Department of Pediatrics [M.W.Q.], Crippled Children's Foundation Research Center [Q.Z., C.S.], LeBonheur Children's Medical Center, University of Tennessee, Memphis, Tennessee 38103, U.S.A.; Department of Pediatrics, University of California, San Diego School of Medicine, La Jolla, California 92103, U.S.A. [D.E.B., H.S., J.F.B., J.C.B.]; Department of Pediatrics and Human Genetics, University of California, Los Angeles, School of Medicine, Los Angeles, California 90095, U.S.A. [R.M.C.]; Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan [T.M., M.F.]; Juntendo University School of Medicine, Bunkyo-ku, Tokyo 113-8421, Japan [K.A.]; and Department of Cardiology, Children's Hospital, Boston, Massachusetts 02115, U.S.A. [J.W.N.]*

## ABSTRACT

Genetic polymorphisms influence the magnitude of the cytokine response after an inflammatory stimulus. To determine whether such polymorphisms might play a role in Kawasaki disease (KD), we analyzed white and Japanese children with KD and control populations for two polymorphic loci in which the A allele is associated with high tumor necrosis factor-α secretion. The lymphotoxin-α+250 A/A genotype was overrepresented among white children with KD compared with controls (0.59 *versus* 0.36; $p = 0.013$). The tumor necrosis factor-α−308 A/G genotype was overrepresented among whites with KD who had coronary artery abnormalities compared with those with normal echocardiograms (0.36 *versus* 0.09; $p = 0.044$). No significant difference was seen at either locus between Japanese children with KD and Japanese controls. The increased frequency of the high secretor alleles in white children with KD suggests that these loci may be related to susceptibility to KD and to outcome after disease. **(*Pediatr Res* 49: 686–690, 2001)**

**Abbreviations:**
**KD,** Kawasaki disease
**CAA,** coronary artery aneurysms
**TNF-α,** tumor necrosis factor-α
**LT-α,** lymphotoxin-α
**sIL-2R,** soluble IL-2 receptor
**IFN-γ,** interferon-γ

KD is an acute systemic vasculitis that affects infants and children and is the leading cause of acquired heart disease in the pediatric age group in the United States and Japan (1, 2). Although the first reports of KD were in Japanese children (3, 4), it is now recognized in children of all races and ethnic groups. Although an infectious agent is suspected, the cause of KD remains unknown. After the acute illness, approximately 15 to 25% of untreated patients will develop damage to the coronary arteries as a result of intense inflammation in the vessel wall (3–5). The high incidence of KD in Japan and among Americans of Japanese descent suggests that these groups may be genetically predisposed to this disease (2, 6). Linkage of different genetic markers (class I and class II MHC antigens, Ig allotypes) with KD has been sought, but only weak associations have been found (7–13).

A number of immunoregulatory changes are observed in children with KD that may contribute to the pathogenesis of the disease (14, 15). Serum levels of several immune mediators are increased, including TNF-α, IL-1, IL-2, IL-6, IL-8, sIL-2R, and IFN-γ (16–19). TNF-α levels are elevated in the majority of children during the acute phase of the disease (17–19) and are highest in children in whom CAA develop (17, 18). *In vitro* studies using vascular endothelial cells have demonstrated that

Received April 27, 2000; accepted October 20, 2000.
Correspondence and reprint requests: Michael W. Quasney, M.D., Ph.D., Division of Critical Care, Department of Pediatrics, Crippled Children's Foundation Research Center, Le Bonheur Children's Medical Center, 50 N. Dunlap, Memphis, TN 38103, U.S.A.; e-mail: mquasney@utmem.edu
Supported in part by the Crippled Children's Foundation (M.W.Q.), the American Heart Association Southeast Affiliate (M.W.Q., Q.Z.), the Kawasaki Disease Research Program at the UCSD School of Medicine, the Pediatric Pharmacology Research Unit (NIH #2U01HD31318), and an NIH training grant (#MO1RR00827 to D.E.B.).

This work was presented in part at the Infectious Disease Society of America, Denver, CO, U.S.A., November 1998; the Sixth International Kawasaki Disease Symposium, Waikaloa, HI, U.S.A., February 1999; and the Society for Pediatric Research, San Francisco, CA, U.S.A., May 1999.

TNF-α, IL-1, and IFN-γ induce the expression of surface antigens that render the cells susceptible to lysis by IgG or IgM antibodies in the sera of children with acute KD (20, 21). TNF-α, therefore, may play an important role in the pathogenesis of the vascular injury in KD.

The stimulus in KD for the increased serum levels of TNF-α remains unknown. The gene coding for TNF-α lies within the MHC on chromosome 6 (22). A number of genetic polymorphisms upstream from the coding sequence for TNF-α have been described and include the nucleotides at LT-α+250, TNF-α−1031, −863, −857, −308, and −238 (Fig. 1) (23–29). The presence of the A allele at the −308 site is associated with elevated TNF-α production in response to endotoxin in whole blood cell cultures (23). The LT-α+250 polymorphic site is approximately 3.2 kb upstream from the transcriptional start site for TNF-α gene and has been associated with higher levels of TNF-α production in stimulated, cultured monocytes (Fig. 1) (27). We tested the hypothesis that children with KD may have a higher frequency of the A allele at the LT-α+250 and the TNF-α−308 sites, each of which are associated with elevated serum levels of TNF-α after an inflammatory stimulus. In addition, we examined the genotypic and allelic frequencies in white and Japanese children with and without KD to determine whether differences in these frequencies were observed across ethnic groups.

## METHODS

***Patients.*** Japanese patients and controls were all born in Japan, and white patients and controls were all born in the United States. Informed consent was obtained from patients or their parents or guardians, and the study was approved by the institutional review boards at the participating institutions. Human experimentation guidelines of the U.S. Department of Health and Human Services and those of the authors' institutions were followed. Efforts were made to recruit patients with CAA so that an association between the polymorphisms and coronary artery abnormalities could be assessed. Children included in the study met the standard clinical criteria for KD (6) and had fever of ≥38°C for 5 or more days and four of the five following criteria: *1*) cervical lymphadenopathy >1.5 cm in diameter; *2*) erythematous rash; *3*) erythematous bulbar conjunctiva without exudate; *4*) erythematous mouth and pharynx, strawberry tongue, or red, cracked lips; and *5*) induration of the hands and feet, erythematous palms and soles, or periungual desquamation. The control populations consisted of adult volunteers with no history of KD, autoimmune diseases, or chronic treatment with anti-inflammatory agents.

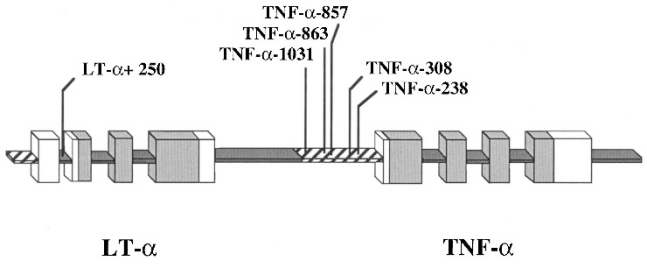


**Fig. 1.** Location of the polymorphic sites affecting TNF-α response. Gray boxes, exons; black boxes, introns; hatched area, promotor; white boxes, 5' and 3' untranslated regions. Not to scale.

***Nomenclature for polymorphisms.*** The LT-α+250 describes the polymorphism 250 bases downstream from the transcriptional start site within the LT-α gene (Fig. 1). The two alleles at this site have been previously designated as TNFB1 when a guanine is present and TNFB2 when an adenine is present (25, 26). The TNF-α−308 describes the polymorphism 308 bases upstream from the transcriptional start site for the TNF-α gene (Fig. 1). Polymorphisms at the TNF-α−308 locus have been previously designated as TNF1 when a guanine is present and TNF2 when an adenine is present (26). We will refer to polymorphisms at these loci as the G allele or the A allele at the LT-α+250 or the TNF-α−308 site.

***Genotypic analysis.*** Whole blood (1.0 mL) was collected, and DNA was extracted using the Genomic DNA Purification Kit (Promega, Madison, WI, U.S.A.). The LT-α+250 polymorphism contains an *Nco*I restriction site when the G allele is present. We amplified a 782-bp fragment in a PCR mixture containing 20 ng of DNA, 20 pmol each of the primers TNFB+250-1 (5'-CCGTGCTTCGTGCTTTGGACTA-3') and TNFB+250-2 (5'-AGAGGGGTGGATGCTTGGGTTC-3') (25), 1 unit of *Taq* polymerase, 1× reaction buffer (Promega), 500 μM each of deoxy-ATP, deoxy-cytidine triphosphate, deoxy-guanosine triphosphate, and deoxy-thymidine triphosphate, and 2.5 mM of $MgCl_2$. Reaction conditions were as follows: 35 cycles of denaturation at 94°C for 30 s, annealing at 68°C for 30 s, and extension at 74°C for 42 s. The amplified DNA was incubated with *Nco*I. The *Nco*I-treated fragments were analyzed by electrophoresis in a 1% agarose gel and visualized by ethidium bromide staining. Interpretation was as follows: a single band at 782 bp identified individuals homozygous for an adenine at the LT-α+250 locus; two bands at 586 and 196 bp identified individuals homozygous for a guanine at the LT-α+250 locus; three bands at 782, 586, and 196 bp identified individuals heterozygous at the LT-α+250 locus.

The region containing the TNF-α−308 locus was amplified using the primers TNFA-308-1 (5'-AGGCAATAGGTTTT-GAGGG*C*CAT-3') and TNFA-308-2 (5'-ACACTCCCCATC-CTCCCTGCT-3') (29). The TNFA-308-1 primer contains 4 bp of the *Nco*I recognition sequence including a mismatched cytosine as shown by the *C* in the TNFA-308-1 primer sequence. This mismatched cytosine allows for creation of an *Nco*I restriction site (CCATGG) when the G allele is present at position −308. A 116-bp PCR product was generated using the following reaction conditions: 35 cycles of denaturation at 95°C for 30 s, annealing at 64°C for 15 s, and extension at 74°C for 15 s. The amplified DNA was incubated with *Nco*I. The *Nco*I-treated fragments were analyzed by electrophoresis in an 8% polyacrylamide gel and visualized by ethidium bromide staining. Interpretation was as follows: a single band at 116 bp identified individuals homozygous for an adenine at the TNF-α−308 locus; two bands at 96 and 20 bp identified individuals homozygous for a guanine at the TNF-α−308 locus; three bands at 116, 96, and 20 bp identified individuals heterozygous at the TNF-α−308 locus.

***Statistics.*** Statistical analysis on genotypic and allelic frequencies was performed using Fisher's exact test. CIs for the odds ratios were determined using the method of Woolf (30). Linkage disequilibrium analysis between the two polymorphisms was performed using Fisher's exact test on the 3 × 3 table of genotypes.

## RESULTS

***Patient characteristics.*** Forty-six white children and 39 Japanese children with KD were enrolled in the study. Fourteen (30%) of the white children and 11 (26%) of the Japanese children had evidence of CAA or dilatation by echocardiography. Of the 46 white patients, 44 were treated with i.v. Ig (2 g/kg), and 12 (27%) had persistent fever 48 h after their initial i.v. Ig infusion and required one or more additional doses. Persistent fever after i.v. Ig administration could not be assessed for the Japanese patients because a variety of different treatment regimens and i.v. Ig doses were used.

***Genotypic and allelic frequencies at the LT-α+250 site.*** The genotypic frequencies of the LT-α+250 polymorphic site located in the first intron of the LT-α gene were compared between children with KD and a healthy control population (Table 1). There was a higher frequency of the A/A genotype among white children with KD compared with the healthy white controls (0.59 *versus* 0.36; $p = 0.013$; odds ratio, 2.5; 95% CI, 1.23 to 5.09). The frequencies of the A and G alleles at the LT-α+250 site in white children with KD were also significantly higher compared with the healthy white controls (A allele, 0.77 *versus* 0.61; $p = 0.009$; odds ratio, 2.2; 95% CI, 1.24 to 3.79; Table 2). In contrast, allelic frequencies in Japanese patients with KD were not different from Japanese controls (A allele, 0.58 *versus* 0.64; $p = 0.51$; odds ratio, 0.76; 95% CI, 0.41 to 1.31). The allelic frequencies for the white and Japanese control populations were similar to previous reports (25, 31, 32).

***Genotypic and allelic frequencies at the TNF-α−308 site and linkage disequilibrium.*** The genotypic and allelic frequencies of the TNF-α−308 polymorphic site located in the promoter region of the gene coding for TNF-α were not different between white and Japanese children with KD and their respective ethnically matched control populations (white, A allele, 0.09 *versus* 0.17; $p = 0.08$; odds ratio, 0.48; 95% CI, 0.21 to 1.07; Japanese, A allele, 0.00 *versus* 0.04; $p = 0.25$; odds ratio, 0.14; 95% CI, 0.01 to 2.71; Tables 1 and 2). Both control populations demonstrated allelic frequencies similar to previously published reports (23, 24, 26). Analyses revealed a significant linkage disequilibrium ($p < 0.000001$) and, in particular, an association between the A allele at the LT-α+250 site and the G allele at the TNF-α−308 site. Thus, an individual who has the A allele at the LT-α+250 site is more likely to have the G allele at the TNF-α−308 site than one would expect by chance alone.

**Table 2.** *Allelic frequencies at the LT-α+250 and TNF-α−308 loci in white and Japanese children with KD compared with healthy control populations*

| Population | LT-α+250 | | TNF-α−308 | |
|---|---|---|---|---|
| | A allele | G allele | A allele | G allele |
| White children with KD | 0.77* | 0.23 | 0.09 | 0.91 |
| White control population | 0.61 | 0.39 | 0.17 | 0.83 |
| Japanese children with KD | 0.58 | 0.42 | 0.00 | 1.00 |
| Japanese control population | 0.64 | 0.36 | 0.04 | 0.96 |

* White children with and without KD (LT-α+250, A allele, 0.77 *versus* 0.61; $p = 0.008$; odds ratio, 2.2; 95% CI, 1.24 to 3.79). All other comparisons not significantly different. Statistical analysis using Fisher's exact test.

***Genotypic and allelic frequencies in children with KD and coronary artery abnormalities or the need for i.v. Ig retreatment.*** An association was found between the frequency of the A/G genotype at the TNF-α−308 locus and the presence of coronary artery abnormalities (0.36 *versus* 0.09; $p = 0.044$; odds ratio, 5.4; 95% CI, 1.07 to 27.01) in white children with KD (Table 3). No association was found between genotypic frequencies at the LT-α+250 locus and coronary artery abnormalities, nor was there an association observed between the genotypic or allelic frequencies at either the LT-α+250 locus or the TNF-α−308 locus and the need for retreatment with i.v. Ig.

## DISCUSSION

The inflammatory reaction produced by a host in response to a stimulus is influenced by a number of factors, including the type of stimulus, the dose of stimulus, and genetic characteristics of the host. The stimulus for the inflammatory reaction in children with KD remains unknown. In this study white children with KD were more likely to have the A/A genotype at the LT-α+250 site compared with a healthy control population and were more likely to have the A/G genotype at the TNF-α−308 site if they developed coronary artery abnormalities. Individuals with either of these genotypes have a higher TNF-α

**Table 1.** *Genotypic frequencies at the LT-α+250 and TNF-α−308 loci in white and Japanese children with KD compared with healthy control populations*

| Population | *n* | LT-α+250 | | | TNF-α−308 | | |
|---|---|---|---|---|---|---|---|
| | | A/A | A/G | G/G | A/A | A/G | G/G |
| White children with KD | 46 | 0.59* | 0.37 | 0.04 | 0.00 | 0.17 | 0.83 |
| White control population | 105 | 0.36 | 0.51 | 0.13 | 0.04 | 0.26 | 0.71 |
| Japanese children with KD | 39 | 0.28 | 0.59 | 0.13 | 0.00 | 0.00 | 1.00 |
| Japanese control population | 39 | 0.44 | 0.41 | 0.15 | 0.00 | 0.08 | 0.92 |

* White children with and without KD (LT-α+250, A/A genotype; $p = 0.013$; odds ratio, 2.5; 95% CI 1.23 to 5.09). All other comparisons not significantly different. Statistical analysis using Fisher's exact test.

**Table 3.** *Genotypic frequencies at the LT-α+250 and TNF-α−308 loci in white and Japanese children with Kawasaki disease and coronary artery abnormalities*

| Population | *n* | LT-α+250 | | | TNF-α−308 | | |
|---|---|---|---|---|---|---|---|
| | | A/A | A/G | G/G | A/A | A/G | G/G |
| Whites with CAA | 14 | 0.50 | 0.43 | 0.07 | 0.00 | 0.36* | 0.64 |
| Whites without CAA | 32 | 0.63 | 0.34 | 0.03 | 0.00 | 0.09 | 0.91 |
| Japanese with CAA | 11 | 0.27 | 0.55 | 0.18 | 0.00 | 0.00 | 1.00 |
| Japanese without CAA | 28 | 0.28 | 0.61 | 0.11 | 0.00 | 0.00 | 1.00 |

* White children with and without CAA (TNF-α−308 A/G genotype; $p = 0.044$; odds ratio, 5.4; 95% CI, 1.07 to 27.01). All other comparisons not significantly different. Statistical analysis using Fisher's exact test.

response than individuals with the G/G genotype at either locus. Thus, development of KD and coronary artery damage in white children may be related to the magnitude of the host's TNF-α response. In Japanese children, in whom the incidence of KD is almost 10-fold higher, no association with these polymorphisms was found. The low prevalence of the A allele in the Japanese population coupled with our small sample size may have precluded our ability to detect a difference.

We assumed *a priori* that there would be genetic heterogeneity between the Japanese and white populations and that different loci might influence disease susceptibility and outcome in the two racial groups. Thus, we did not choose to pool the two data sets. We did not detect a difference in genotype frequencies between the Japanese and white control samples, indicating that pooling would be possible. However, there were significant differences in genotype frequencies, and hence genotypic heterogeneity, among the cases for both the LT-α+250 ($p = 0.014$) and TNF-α−308 ($p = 0.007$) sites.

Certain polymorphisms within the promoter region of the gene coding for TNF-α are associated with elevated TNF-α levels when peripheral blood mononuclear cells are treated with concanavalin A (24). Their positions relative to the TNF-α transcriptional start site are −1031C, −863A, −857A, −308A, and −238A. The presence of the A allele at the −308 site has also been shown to result in elevated TNF-α production in response to endotoxin in whole blood cell cultures (23). In a recent study, these loci were compared between Japanese children with KD and healthy Japanese controls, and, as in the present study, no association was found between high secretor genotypes and KD in Japanese children (28). Japanese patients with KD may still have a TNF-α high secretor phenotype, despite the failure to demonstrate a relationship between these loci and susceptibility to disease. The regulation of the TNF-α response to inflammation is controlled both at transcriptional and posttranscriptional levels and each may be influenced by independent genetic factors. A major factor in the determination of TNF-α levels is stabilization of TNF-α mRNA by AU-rich domains in the 3' untranslated region of the mRNA (33–36)). The genetics of this posttranscriptional control mechanism are unknown. It is possible that polymorphisms influencing TNF-α mRNA stability are also important in KD and that these loci are influential in the Japanese population in determining disease susceptibility. A more complete understanding of the genetic influences on disease susceptibility must await a refinement in our knowledge of the genetics of the TNF-α response.

The LT-α+250 polymorphic site has been associated with human disease in only one other instance. Stuber *et al.* (25) demonstrated that the frequency of the A allele at the LT-α+250 site was higher in septic patients who died compared with survivors. The serum levels of TNF-α correlated well with the genotype; those patients with the A/A genotype had higher serum TNF-α levels whereas those with the A/G or G/G genotypes had lower serum TNF-α levels. Our study is the second report of an association between a human disease and the LT-α+250 polymorphism.

A limitation of our analyses is the small sample size coupled with the heterogeneity of the white population. We cannot exclude that population stratification alone could account for the observed differences in allele frequency. This preliminary study suggests that further testing of our hypothesis is warranted. We are currently exploring the difference in transmission frequencies of the A alleles at these polymorphic sites by the transmission disequilibrium test. By analyzing triads of affected patients and their parents, we will further test whether these alleles, or alleles in linkage disequilibrium with these alleles, are implicated in disease susceptibility or disease outcome.

An increased frequency of the TNF-α−308 A allele has been associated with poor outcome after certain infections. An association between serum TNF-α levels and mortality in meningococcal meningitis has been observed (37), and children with the A allele at the TNF-α−308 site have a worse outcome after meningococcal infection (38). Similar associations have been observed in individuals with malaria; those with higher TNF-α levels had a worse outcome (39), and the TNF-α−308 A allele site was overrepresented among patients with cerebral malaria and among those who died of malaria (40).

A genetic predisposition for KD is suggested by the higher prevalence of the disease among Japanese and Americans of Japanese descent. Several studies have demonstrated weak associations between KD and haplotypes in the MHC locus, suggesting that several genes within this locus may play a role in the pathogenesis of KD. For example, an association of the HLA antigens, HLA-Bw15 and Bw22, with KD was found in Japan (7), although not all studies support this finding (8). No association with HLA-Bw22 or class II antigens was observed in children with KD in the United States (9, 10, 12). Rather, a weak association with HLA-Bw51 and KD was observed in the United States (9) and Israel (11). The specific sites within these loci that are more common in children with KD have yet to be determined. Certain Ig allotypic markers were overrepresented among white but not Japanese KD patients (13). Taken together, no clear genetic influence on disease susceptibility emerges from these data.

Our findings suggest an association between the frequency of the A allele at the TNF-α−308 locus and the presence of CAA in white children. This raises the possibility that the vascular injury observed in children with KD is the result of a local inflammatory reaction in children genetically predisposed to have a more vigorous inflammatory response. If this association is supported by future studies, it may be possible to use genotyping to identify children who would benefit from closer monitoring and more-directed therapies to prevent vascular damage. Larger numbers of patients with and without aneurysms must be studied to more accurately assess the association between the TNF-α−308 allele and development of coronary artery abnormalities. The failure to find an association between the A allele at the TNF-α−308 locus and the presence of CAA in Japanese children may be related to our small sample size and the extremely low frequency of the A allele in the Japanese population. It may also be that the A allele at the LT-α+250 is in linkage disequilibrium in whites but not Japanese, with a more important TNF-α regulatory allele not examined in these analyses.

The mechanisms by which the polymorphic sites at positions LT-$\alpha$+250 and TNF-$\alpha$−308 affect TNF-$\alpha$ levels are unknown. The polymorphism at the TNF-$\alpha$−308 site lies within the promoter region for the TNF-$\alpha$ gene and may alter the binding of transcription factors. Studies with the TNF-$\alpha$ promoter linked to a chloramphenicol acetyltransferase reporter gene have demonstrated that the A allele at TNF-$\alpha$−308 site is associated with higher constitutive and inducible levels of transcription than the more common G allele (41). Kroeger *et al.* (42) demonstrated, furthermore, a difference in the ability of this region to bind nuclear proteins. The LT-$\alpha$+250 site, on the other hand, is approximately 3.2 kb from the transcriptional start site for TNF-$\alpha$. *cis*-acting transcriptional regulatory elements, such as enhancers, are known to act over such distances. Current studies are underway in our laboratory to identify the molecular mechanisms by which this region affects serum levels of TNF-$\alpha$.

In summary, white, but not Japanese, children with KD have a higher frequency than a control population of the A/A genotype in one of two polymorphic sites in the 5'-flanking region of the gene coding for TNF-$\alpha$. White children who are homozygous for A/A at the LT-$\alpha$+250 site may be genetically predisposed to KD. Furthermore, white children with the A allele at the TNF-$\alpha$−308 site may be at greater risk for the development of coronary artery abnormalities. These findings support the hypothesis that genetic polymorphisms that influence the host immune response may play an important role in susceptibility to KD and to disease outcome.

***Acknowledgments.*** The authors thank Shelley Bakalis, Chisato Shimizu, and Sachiko Suematsu for collecting and extracting the DNA samples and Shoichi Oyama, Saiseiki Kawaguchi Hospital, for contributing samples.

## REFERENCES

1. Taubert KA, Rowley AH, Shulman ST 1991 Nationwide survey of Kawasaki disease and acute rheumatic fever. J Pediatr 119:279–282
2. Yanagawa H, Yashiro M, Nakamura Y, Kawasaki T, Kato H 1995 Results of 12 nationwide epidemiological incidence surveys of Kawasaki disease in Japan. Arch Pediatr Adolesc Med 149:779–783
3. Kawasaki T, Kosaki F, Okawa S, Shigematsu I, Yanagawa H 1974 A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. Pediatrics 54:271–276
4. Melish ME, Hicks RM, Larson EJ 1976 Mucocutaneous lymph node syndrome in the United States. Am J Dis Child 130:599–607
5. Kato H, Koike S, Yamamoto M, Ito Y, Yano E 1975 Coronary aneurysms in infants and young children with acute febrile mucocutaneous lymph node syndrome. J Pediatr 86:892–898
6. Morens DM, Anderson LJ, Hurwitz ES 1980 National surveillance of Kawasaki disease. Pediatrics 65:21–25
7. Kato S, Kimura M, Tsuji K, Kusakawa S, Asai T, Juji T, Kawasaki T 1978 HLA antigens in Kawasaki disease. Pediatrics 61:252–255
8. Matsuda I, Hattori S, Nagata N, Fruse A, Nambu H 1977 HLA antigens in mucocutaneous lymph node syndrome. Am J Dis Child 131:1417–1418
9. Krensky AM, Berenberg W, Shanely K, Yunis EJ 1981 HLA antigens in mucocutaneous lymph node syndrome in New England. Pediatrics 67:741–743
10. Krensky AM, Grady S, Shanely KM, Berenberg W, Yunis EJ 1983 Epidemic and endemic HLA-B and DR associations in mucocutaneous lymph node syndrome in New England. Hum Immunol 6:75–77
11. Keren G, Danon YL, Orgard S, Kalt R, Gazit E 1982 HLA-Bw51 is increased in mucocutaneous lymph node syndrome in Israel patients. Tissue Antigens 20:144–146
12. Fildes N, Burns JC, Newburger JW, Kiltz W, Begovich AB 1992 The HLA class II region and susceptibility to Kawasaki disease. Tissue Antigens 39:99–101
13. Shulman ST, Melish M, Inoue O, Kato H, Tomita S 1993 Immunoglobulin allotypic markers in Kawasaki disease. J Pediatr 122:84–86
14. Leung DYM 1991 Immunologic aspects of Kawasaki disease: implications for pathogenesis and therapy. Clin Cardiol 14:11–15
15. Lin CY, Hwang B 1987 Serial immunologic studies in patients with mucocutaneous lymph node syndrome (Kawasaki disease). Ann Allergy 59:291–297
16. Lin CY, Lin CC, Hwang B, Chiang BN 1991 The changes of interleukin-2, tumor necrosis factor and gamma-interferon production among patients with Kawasaki disease. Eur J Pediatr 150:179–182
17. Maury CP, Salo E, Pelkonen P 1989 Elevated circulating tumor necrosis factor-alpha in patients with Kawasaki disease. J Lab Clin Med 113:651–654
18. Matsubara T, Furukawa S, Yabuta K 1990 Serum levels of tumor necrosis factor, interleukin 2 receptor, and interferon-gamma in Kawasaki disease involved coronary-artery lesions. Clin Immunol Immunopathol 56:29–36
19. Lang BA, Silverman ED, Laxer RM, Lau AS 1989 Spontaneous tumor necrosis factor production in Kawasaki disease. J Pediatr 115:939–943
20. Leung DYM, Collins T, Lapierre LA, Geha RS, Pober JS 1986 Immunoglobulin M antibodies present in the acute phase of Kawasaki syndrome lyse cultured vascular endothelial cells stimulated by gamma interferon. J Clin Invest 77:1428–1435
21. Leung DYM, Geha RS, Newberger JW, Burns JC, Fiers W, Lapierre LA, Pober JS 1986 Two monokines, interleukin-1 and tumor necrosis factor, render cultured vascular endothelial cells susceptible to lysis by antibodies circulating during Kawasaki disease. J Exp Med 164:1958–1972
22. Nedwin GE, Naylor SL, Sakaguchi AY, Smith D, Jarrett-Nedwin J, Pennica D, Goeddel DV, Gray PW 1985 Human lymphotoxin and tumor necrosis factor genes: structure, homology and chromosomal localization. Nucleic Acids Res 13:6361–6373
23. Louis E, Franchimont D, Piron A, Gevaert Y, Schaaf-Lafontaine N, Roland S, Mahieu P, Malaise M De Groote D, Louis R, Belaiche J 1998 Tumour necrosis factor (TNF) gene polymorphism influences TNF-$\alpha$ production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. Clin Exp Immunol 113:401–406
24. Higuchi T, Seki N, Kamizono S, Yamada A, Kimura A, Kato H, Itoh K 1998 Polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)-$\alpha$ gene in Japanese. Tissue Antigens 51:605–612
25. Stuber F, Petersen M, Bokelmann F, Schade U 1996 A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor concentrations and outcome of patients with severe sepsis. Crit Care Med 24:381–384
26. Stuber F, Udalova IA, Book M, Drutskaya LN, Kuprash DV, Turetskaya RL, Schade FU, Nedospasov 1996 −308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. J Inflamm 46:42–50
27. Pociot F, Briant L, Jongeneel CV, Molvig J, Worsaae H, Abbal M, Thomsen M, Nerup J, Cambon-Thomsen A 1993 Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-$\alpha$ and TNF-$\beta$ by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. Eur J Immunol 23:224–231
28. Kamizono S, Yamada A, Higuchi T, Kato H, Itoh K 1999 Analysis of tumor necrosis factor-alpha production and polymorphisms of the tumor necrosis factor-alpha gene in individuals with a history of Kawasaki disease. Pediatr Int 41:341–345
29. Wilson AG, di Giovine FS, Blakemore A, Duff GW 1992 Single base polymorphism in the human tumor necrosis factor alpha (TNF) gene detectable by *Nco*I restriction of PCR product. Hum Mol Genet 1:353 (abstr)
30. Rosner B 1995 Fundamentals of Biostatistics. Duxberry Press, Boston, pp 368–369
31. Fugger L, Morling N, Ryder LP, Platz P, Georgsen J, Jakobsen BK, Svejgaard A, Dalhoff K, Ranek L 1989 *Nco* I restriction fragment length polymorphism (RFLP) of the tumor necrosis factor (TNF$\alpha$) region in primary biliary cirrhosis and in healthy Danes. Scand J Immunol 30:185–189
32. Shimura T, Hagihara M, Takebe K, Munkhbat B, Ogoshi K, Mitomi T, Nagamachi Y, Tsuji K 1995 10.5-kb homozygote of tumor necrosis factor-beta gene is associated with a better prognosis in gastric cancer patients. Cancer 75:1450–1453
33. Wang E, Ma WJ, Aghajanian C, Spriggs DR 1997 Posttranscriptional regulation of protein expression in human epithelial carcinoma cells by adenine-uridine-rich elements in the 3'-untranslated region of tumor necrosis factor-alpha. Cancer Res 57:5426–5433
34. Kim Y, Rus HG, Fisher SN, Pitha PM, Shin ML 1996 Binding of a protein to an AU-rich domain of tumor necrosis factor alpha mRNA as a 35kDa complex and its regulation in primary rat astrocytes. Biochem J 316:455–460
35. Furse RK, Kodys K, Zhu D, Miller-Graziano CL 1997 Increased monocyte TNF-$\alpha$ message stability contributes to trauma patients' increased TNF production. J Leukoc Biol 62:524–534
36. Leverkus M, Yaar M, Eller MS, Tang EH, Gilchrest BA 1998 Post-transcriptional regulation of UV induced TNF-$\alpha$ expression. J Invest Dermatol 110:353–357
37. Waage A, Halstensen A, Espevik T 1987 Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet 1:355–357
38. Nadel S, Newport MJ, Booy R, Levin M 1996 Variation in the tumor necrosis factor-alpha gene promoter region may be associated with death from meningococcal disease. J Infect Dis 174:878–880
39. Grau GE, Taylor TE, Molyneux ME, Wirima JJ, Vassalli P, Hommel M, Lambe PH 1989 Tumor necrosis factor and disease severity in children with falciparum malaria. N Engl J Med 320:1586–1591
40. McGuire W, Hill AVS, Allsopp CEM, Greenwood BM, Kwjatkowski D 1994 Variation in the TNF-$\alpha$ promoter region associated with susceptibility to cerebral malaria. Nature 371:508–511
41. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW 1997 Effects of a polymorphism in the human tumor necrosis factor $\alpha$ promoter on transcriptional activation. Proc Natl Acad Sci U S A 94:3195–3199
42. Kroeger KM, Carville KS, Lawrence LJ 1997 The −308 tumor necrosis factor-$\alpha$ promoter polymorphism effects transcription. Mol Immunol 34:391–399