We investigated IgG antibodies against Mycobacterium bovis heat shock protein 65 (M. bovis Hsp65) fragments produced by cleavage with cyanogen bromide (CNBr) in 10 healthy controls, 11 patients with juvenile idiopathic arthritis (JIA) and 10 children with various malignant and non-malignant diseases before allogeneic stem cell transplantation (SCT) by using Western blotting (WB). CNBr cleaved M. bovis Hsp65 to three larger fragments: Pl-163, molecular weight (Mw) 17.1 kDa; P191-285, Mw 10.4 kDa and P290-534, Mw 25.3 kDa. Humoral reactivity against M. bovis Hsp65 derived fragments differed on a case-by-case basis. The sera either of JIA patients ar those befare SCT reacted with individual M. bovis Hsp65 fragments more frequently when compared with healthy controls. While IgG anti-Hsp65 antibody levels showed no significant differences between the small studied cohorts, significantly higher levels of antibodies against M. bovis Hsp65 epitopes were observed in patients befare SCT and JIA patients when compared with healthy controls. Comparing WB reflectance densities (DRl and DR2), significantly elevated antibodies against Pl-163 (DRl: p=0.014; DR2: p=0.022) and P290-534 (DRl: p=0.009; DR2: p=0.003) epitopes were found in patients befare SCT. Similarly, significantly increased DRl and DR2 values of antibodies against P1-l63 (DRl: p=0.Ol8; DR2: p=0.006) and P290-534 (DRl: p=0.05; DR2: p= 0.04) epitopes were detected in JIA patients. The immune system of each individua I would react to different epitopes of M. bovis BCG Hsp65 immunodominant antigen. An increased humoral response against individual Hsp65 derived fragments in a cohort of patients before SCT might be explained by frequent infection in immunocompromised patients suffering from different malignant and non-malignant diseases.