ABSTRACT

Transfusion-associated microchimerism (TA-MC) is the stable persistence of an allogeneic cell population, resulting from allogeneic blood transfusion (often resulting after trauma). Currently TA-MC is not completely understood, needing further study to reveal its underlying mechanism. This article reviews the immune tolerance mechanism of TA-MC; factors which lead to the appearance of TA-MC; clinical implications of microchimerism; and the latest diagnostic methods of TA-MC.

Keywords: transfusion–associated microchimerism, blood transfusion, trauma

TRANSFUSION-ASSOCIATED MICROCHIMERISM (TA-MC)

The word "chimera" first appeared in Homer’s Iliad, describing a fire-breathing beast. This beast had the head of lion, the body of goat and the tail of snake and was believed to be a sign of disaster[1]. In the field of science, "chimera" is a hybrid single group consisting of genetically different cells. "Microchimerism" is the presence of a small number of heterogeneous cells (<5%) in a genetically different host individual. There are a variety of conditions that can cause microchimerism, including pregnancies where a mother retains a small number of fetus cells, and the fetus is born with a few cells genetically different from their mothers[2,3], solid organ and hematopoietic stem cell transplantation[4] and blood transfusion[5]. As its name implies, transfusion–associated microchimerism (TA-MC) is the persistent allogeneic exposure to foreign cells caused by blood transfusion, particularly follow–

Research concerning TA-MC has gone through three landmark phases: ① In the 1970s, Schechter et al. demonstrated karyotype evidence of donor leukocyte proliferation in recipients within 7 days (failing to increase over 7 days) after transfusion[7,8]. ② Lee et al. conducted a case–control study which replicated early experience: persistent TA-MC appeared in transfused patients with trauma within 20 years[9]. ③ Blood transfusions in patients with severe traumatic injuries result in a high incidence (20%~40%) of TA-MC persistence over a long time[10].

TA–MC FOLLOWING TRAUMATIC INJURY

Many studies observed that TA–MC is closely related to blood transfusion after traumatic injury. PCR HLA typing and mixed leukocyte reaction (MLR) were performed by Lee et al. finding that 7 out of 10 female trauma patients exhibited a multilineage persistence of male donor white blood cells from 6 months to 1.5 years[9]. Utter et al. found post trauma transfusion is associated with evidence of microchimerism among over half of recipients (24 out of 45 subjects, 53%)[6]. Additionally, Utter et al. also found that TA–MC is a common phenomenon post transfusion after the occurrence of trauma[11]. In brief, the percentage of TA–MC in civilian trauma patients

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receiving allogenic red blood cells (RBCs) transfusions is about 20%-40%. Dunne et al. explored that the incidence of TA–MC in combat casualties receiving fresh whole blood (3 of 6, 50%), platelets (4 of 8, 50%) and RBCs (3 of 8, 38%), suggesting that TA–MC in transfused soldiers is very common[10].

THE MECHANISM OF TA–MC

The mechanism of TA–MC has not yet been fully revealed. However, changes in the immune system caused by trauma and blood transfusion may be the underlying cause[11]. Short-term immunosuppression and complications due to infections are major causes of death in post trauma patients[12]. Early studies have shown that trauma and stimulation impair innate and adaptive immunity function, involving T cells, B cells, and monocytes. Studies in vivo have shown that Th2 type cytokines such as IL–4 and IL–10 increase while mice down-regulate Th1 type cytokines (IL–2, IFN–γ) and decrease T cell proliferative response when stimulated with phytohemagglutinin (PHA), pokeweed mitogen (PWM) or bacterial lipopolysaccharide (LPS) for 5 to 10 days after trauma[13-15], which is consistent with findings in humans[16,17]. In addition, IgG/IgM reduced expression. Although the monocytes increased, they inhibited the secretion of IFN–γ and expression of HLA–DR. Early studies also indicated that macrophage up-regulated the expression of prostaglandin E2[18], IL–6[19] and IL–10[20] following the inhibition of T cells; Toll–like receptors (TLR)–2 and TLR–4 induced the augment of IL–1, IL–6 and TNF–α secretion by macrophages in the dysregulation of innate immune response[21]; and IL–10 showed an elevated expression caused by macrophages after severe trauma[22]. At the molecular level, peripheral blood mononuclear cells secrete less NF–κB, which impairs the activation of T cells[23]. All of the above studies focused on the inhibition of Th2 cytokines to T cells’ immune responses following trauma. Therefore, post-trauma immune system disorder may explain why TA–MC happens to trauma patients after receiving blood transfusions. Traumatic injury and blood transfusion are critical immune stimuli which induce immune regulation to form a micro-environment for the development of TA–MC. In a case–control study, TA–MC patients who underwent post trauma transfusion had a weak lymphocyte response compared to patients with trauma, but had not undergone blood transfusion. The above studies are limited to the analysis of "short–term TA–MC". However, how these chimeras keep stable in the host’s immune system for decades is still unclear. One explanation is that the host’s immune tolerance may induce body refractory to the chimeras[9].

THE FACTORS WHICH LEAD TO THE APPEARANCE OF TA–MC

To date, almost all studies have shown that TA–MC only occurs in patients who have been transfused after traumatic injury. However, not all post–traumatic blood transfusions cause TA–MC, so further research is needed to find other related factors, such as: HLA compatibility, severity of trauma, variability of transfusion products, etc. Ultimately, the development of TA–MC depends on how these factors influence immune responses[1]. In recent years, research progress concerning the factors affecting the formation of TA–MC is exemplified by the following examples:

(1) In a prospective cohort study, Bloch et al. set out to determine if transfusion in the peripartum period results in lasting high–level TA–MC. Evidence of transient TA–MC was found in 2 of 22 women who received blood transfusion within 48 hours. However, no persistent TA–MC was detected at 6 weeks or 6 months post transfusion[24].

(2) A population–based case–control study conducted by Müller et al. found that microchimerism in young expectant mothers may originate from transfusion during discontinued pregnancy[25].

(3) A study conducted by Sanchez et al. aimed to evaluate whether 409 adult and pediatric females receiving leuko–reduced and mostly irradiated allogeneic RBC and platelet transfusions would develop TA–MC. They found that 40 of 207 (19%) of adult and 44 of 202 (22%) pediatric female blood recipients went on to develop low level non–persistent MC, demonstrating that leuko–reduced and mostly irradiated allogeneic RBC and platelet transfusions are able to prevent the adult and pediatric recipient from developing enduring TA–MC[26]. Conversely, from a study of Australian trauma patients (n=36) who received RBC transfusions from 2000 to 2012: 9 of 55 (16.3%) patients transfused with non–leuko-depleted blood components and 3 of 31 (9.6%) transfused with leukodepleted RBC units developed persistent TA–MC. Although non–leuko-depleted blood components are now commonly used in Australia, clinical results show that the incidence of TA–MC has not changed significantly[27]. Notably, half of the patients included in the above study were recorded as splenic injury or required splenectomy during transfusion. From this, we believe the spleen may play a pivotal role in filtering abnormal cells and producing antibodies and lymphocytes. In a suggested splenic injury scenario, the donor cells may still be able to escape the host’s immune system to form TA–MC, even if the recipient...
has received non–leukodepleted blood components.

**CLINICAL IMPLICATON OF TA–MC**

The negative clinical significance of “microchimerism” is thought to be related to autoimmune diseases\[20\]. Studies in support of this have found that fetal chimeric cells are commonly found in systemic lupus erythematosus (SLE) females with traumas. Significant differences were found between SLE traumatic patients and healthy controls \( P < 0.001 \), while no statistically significant differences were found between traumatic SLE female patients and non–traumatic SLE female patients \( P= 0.036 \)[29]. Therefore, the relationship between autoimmune diseases and micro–chimeras appears to be of the “chicken and egg” variety, where it seems impossible to determine a clear relationship\[30\]. Micro–chimerism may have some positive aspects: ① "Repair hypothesis" suggests chimeric cells are capable of differentiating into parenchymal cells in response to tissue injury, aiding ability for multi–lineage differentiation\[29\]. ② Prenatal genetic diagnosis, which currently relies on invasive sampling e.g. chorionic villus biopsy and amniocentesis, both of which increase the risk of abortion. If fetal chimeric cells or cell–free DNA in the peripheral circulation of pregnant women can be accurately detected, pregnant women may be able to avoid high–risk sampling\[31,32\].

**DIAGNOSTIC ANALYSIS METHOD FOR TA–MC**

Early technologies for detecting TA–MC rely mainly on: ① PCR targeting the Y–chromosome, which is a sex–based method. ② Quantitative real–time PCR, which has greatly improved the detection sensitivity and specificity of TA–MC\[33\]. ③ HLA–DR alleles assay, which recognizes insertion/deletion (In–Del) polymorphisms\[34\]. The above methods’ detailed instructions have been previously published. This review, however, describes a method for sorting microchimerism in blood cells by flow cytometry, based on human HLA antigen. The method outlined below was developed by Drabbel's\[35\] and Eikmans et al.'s\[36\], who established the most efficient separation method, with a sorting ratio as low as 0.01%. Here two different human monoclonal HLA antibodies were used in the assay, one for the chimeric cells, the other targeted background cells. After successful isolation, DNA analysis was used to detect the specificity of the HLA allele and Y–chromosome–RT–qPCR was performed to verify the purity of the isolated cell population. When compared with pre–treated samples, chimeric DNA markers’ PCR signals were significantly enhanced on positive population. Due to these proper–ties, this method was successfully applied to isolate maternal micro–chimera cells from umbilical cord mononuclear cells. This new technology is able to maximize the enrichment of micro chimeric cells that may exist in the host, opening a new horizon for the study of micro chimeric cell’s phenotype and function.

In summary, TA–MC research is still in its infancy, and scientists are still required to conduct further research to explore both its negative, positive effects and deeper mechanism, in order that it may be regulated or manipulated to the benefit of the patient.

**References**


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