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Cytokine-induced killer cell therapy-associated idiopathic thrombocytopenic purpura: rare but noteworthy

Idiopathic thrombocytopenic purpura (ITP) is characterized by a diminished platelet count, an autoimmune condition with antibodies against platelets and an increased tendency to bleed. The association between ITP and solid tumors is uncommon. Cytokine-induced killer (CIK) cell therapy is a well tolerated and promising cancer treatment with minimal toxicity. For the first time, CIK cell therapy was reported to be followed by ITP. The mechanism through which CIK induces ITP remains unclear. Imbalanced ratio of Th cells, decreased numbers or impaired function of Treg cells and excessive secretion of cytokines inducing abnormal activation of B cells may be among the possible reasons. Therefore, a better understanding of this rare condition will require further investigation of these cases.

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Keywords: CIK • immunotherapy • ITP • solid tumor

Background

The current incidence rate of idiopathic thrombocytopenic purpura (ITP) in adults is 3.3 per 100,000 patients each year and the median diagnosed age is 56–60 [1]. ITP is a syndrome with poorly understood pathogenesis and most cases are asymptomatic [2]. If the platelet count falls below $20 \times 10^9/L$, extremely low platelet count may lead a petechiae of skin and bleeding in the mucous membranes or internal organs [3]. There are no definite clinical symptoms or laboratory parameters to establish diagnosis for ITP [4]. Therefore, the diagnosis of ITP requires the exclusion of other etiologies, which is often difficult [4,5]. Antibodies against platelets may be detectable in approximately 60% of ITP patients [6]. High levels of immunoglobulin G (IgG) or immunoglobulin M (IgM) antiplatelet antibodies are often found in patient's peripheral blood plasma [7]. Examination of bone marrow may show megakaryocytes to be normal or increased, and occasionally, an increased

number of immature megakaryocytes are present. Ultra-structural examination may provide some evidence of megakaryocyte apoptosis [8].

Cancer, the leading cause of death worldwide, has seen increased incidence in the past decades [9]. However, the association between ITP and solid tumors has been rarely described. We collected all 79 published cases of ITP associated with solid tumors. The most common cases of tumor associated ITP were found in breast [10–13], lung [14–18] and urinary system [19–22].

Recently, immunotherapy has emerged as a promising approach for cancer treatment [23]. Cytokine-induced killer (CIK) cells are a heterogeneous type of efficient immune effector cells with potent stronger and broader spectrum antitumor activity [24]. Studies showed that CIK cell therapy was minimally toxic, well tolerated and did not have any dose-limiting toxicities [25]. To our knowledge, incidence of ITP during CIK cell therapy has not been reported.

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In our hospital, almost 3500 patients received more than 15,000 transfusions of autologous CIK cell therapy during the past 6 years. Two cases of ITP associated with CIK cell therapy were encountered in our department. This may be the first case report examining the correlation between ITP and CIK cell therapy in solid tumors.

Case presentation

Case one

A 41-year-old woman, presenting with recurrent episodes of left chest fullness and cough without sputum, was admitted to our hospital in August of 2010. Clinical examination found reduction of left lung respiratory sounds and small rhonchi by auscultation. No other system disorders and medication history were previously reported. Pathologic diagnosis was lung adenocarcinoma and TNM stage is IV (T4N2M1a). The patient accepted GP regimen chemotherapy as initial management (gemcitabine 1250 mg/m² days 1 and 8; cisplatin 50 mg/m² days 1, 8, 15 by intrapleural injection). After one cycle of chemotherapy, the patient's symptoms were alleviated to some extent and the pleural effusion disappeared by CT scan findings, however, the mass and enlarged mediastinal lymph nodes were almost the same as before (Figures 1 & 2). The following therapy was adjusted to TP regimen chemotherapy plus CIK immunotherapy (paclitaxel 175 mg/m² day 1; cisplatin 25 mg/m² days 2–4). CIK cells were prepared as before: the peripheral blood mononuclear cells were isolated from 50 ml blood of the patient 3 days prior to chemotherapy, cultured in medium (GT-T551, Takara) with the presence of human IFN- γ , IL-2 and anti-CD3 monoclonal antibody (MAb). The cells were incubated in a humidified atmosphere with 5% CO₂ at 37°C. On days 12–14, the cells were harvested and analyzed for phenotype and cytotoxicity. Then CIK cells were ready to be transfused in 2–4 days after chemotherapy. The phenotype analysis of the patient's CIK cells was presented as Table 1. After two cycles of chemotherapy combined with CIK cell therapy, the patient's symptoms were alleviated noticeably. CT scan revealed that the mass was diminished significantly (Figure 3). The objective response rate was partial remission (PR), valued by Response Evaluation Criteria in Solid Tumors (RECIST). Unavoidably, every cycle of chemotherapy could cause grade 2 myelosuppression such as neutropenia and thrombocytopenia. Moreover, the platelet count was reduced significantly to 13×10^9 /l accompanied by scattered petechiae on the skin of bilateral lower limb after the third-cycle therapy. Two weeks later, the total wright blood cells were returned to a normal level by using granulocyte colony stimulating factors (G-CSF). Otherwise, the platelet

count remained less than 30×10^9 /l and this situation lasted for two months. 63 days later, the platelet count hovered around 18×10^9 /l, still low and as other conditions might exist, ITP was especially doubted. Bone marrow aspiration and biopsy was performed. The results revealed too few platelets, the megakaryocyte count was 31 and of a predominantly granular type. Advanced examination of platelet antibody (PA) indicated PAIgM was higher than normal (Table 2). Diagnosis of ITP was confirmed. Methylprednisolone was used with the dose of 80 mg per day. After 5 consecutive days usage, the patient's platelet count rose to 129×10^9 /l. With the corticosteroid dosage tapered off, platelet count maintained in the normal range.

Case two

In December 2012, a 63-year-old female presented to our hospital with the symptoms of anorexia and dysuria. Physical examination was negative. No system diseases and drug history were previously reported. Abdominal ultrasound showed a large mass (124 mm x 84 mm) in the right kidney. CT scan indicated a large soft tissue lump in the middle zone of the right kidney may be considered renal carcinoma. Multiple enlarged perirenal lymph nodes were recognized as metastases. In addition, multinodular pulmonary lesions were observed, another site of metastasis. Bone scintigraphy results did not conclusively show a metastatic lesion on the second lumbar vertebra. There were no indications for nephrectomy and the patient denied kidney biopsy. Final pathological results due to a progressively enlarged neoplasm located at the mandibular central incisor gingiva. Pathology confirmed the tumor originated from mesenchymal tissue and epithelial components of the kidney. The patient underwent three courses of CIK cell therapy, on 16th January, 31st January and 11th March in 2013. CIK cell culture protocols were the same as described above. During every course 3.9 – 4.5×10^9 CIK cells were transfused to the patient (Table 3). The primary complete blood count test on 24 January of 2013 revealed that platelet count was 74×10^9 /l. However, the platelet count decreased to 40×10^9 /l when the neoplasm ablation was done on 1st March, 2013. 5 days later, a mass reappeared in the same location and scattered hemorrhagic spots on bilateral inner thighs were observed. Advanced examination of antiplatelet antibody levels showed the PAIgG level was approximately triple the upper limit of normal (Table 4). Bone marrow aspiration and biopsy was performed on 6th March, which revealed aporadic platelets, accompanied by an increased number of megakaryocytes, most of which were granular type. We reexamined the platelet count on 11th March and

it had risen to $74 \times 10^9/l$. The diagnosis of ITP was made and an undeniable link between ITP and CIK cell transfusion was considered. The CIK cell therapy was terminated and the patient refused to accept further antitumor therapies.

Conclusion

ITP is an autoimmune condition with antibodies detectable against several platelet surface antigens. Due to the destruction of platelets by these antibodies (especially IgG), platelet count is low. Its onset can be primary or secondary to infections, tumors or other factors. Studies suggest ITP patients have an increased risk of thrombosis, as the seriously decreased platelet count correlates with the development of thrombosis [26,27]. There is no direct relationship between platelet count and bleeding severity [27].

Laboratory studies showed detectable antiplatelet antibodies in 60% of patients. The antibodies are often derived from auto-reactive B cells and against platelet membrane glycoproteins IIb-IIIa or Ib-IX, which are the type of IgG [28]. Thrombocytopenia occurs due to the Fc-dependent platelet clearance in the spleen and megakaryocyte apoptosis. Furthermore, the antibodies target the megakaryocytes, impairing platelet production and finally leading to diminished peripheral platelet count [29,30]. Measurement of platelet associated antibodies offers 78–92% specificity of ITP diagnosis, however, the sensitivity ranges from 49 to 66% [31]. Thus, the positive predictive value for diagnostic utility remains limited.

CIK cells are a heterogeneous population of immune effector cells which feature mixed T and Natural Killer (NK) cells. Predominantly, the phenotype is $CD3^+CD56^+$ T lymphocytes. Recent clinical research, primarily in Asia, has shown CIK cell immunotherapy can significantly improve progression-free survival (PFS) and overall survival (OS) in multiple types of solid tumor, especially in patients with liver, gastric, kidney and lung cancers [32–36].

Clinical studies indicated the most common adverse events (AEs) were transient fever, chills and fatigue [24,37]. Further side effects included mild hypotension, headache, nausea, vomiting, malaise, dizziness, conjunctivitis, keratitis, rash and injection site infection [24,38]. Most patients with AEs needed no medical interventions. The patients with severe AEs only needed treatment with simple symptomatic measures such as anti-inflammatory, anti-emetic and antibacterial medications [33]. The rare but severe AEs involved anaphylactic shock, tumor lysis syndrome, arthralgia, acute and chronic graft versus host disease (GVHD) [24,37]. No CIK cell therapy associated with ITP cases are reported yet.

Figure 1. The primary image of the patient's CT scan.

In our case presentations, two women were diagnosed lung cancer and renal malignant cancer,

Figure 2. CT scan image after the first cycle of chemotherapy.

Figure 3. CT scan image after the second cycle of chemotherapy combined with cytokine-induced killer cell therapy.

respectively. Both had nearly normal platelet counts and no bleeding symptoms at their first visit. In other words, symptoms of ITP did not precede tumor diagnosis. However, when diminished platelet count, scattered petechiae and increased platelet antibodies appeared after CIK cell therapy, ITP was diagnosed. It is worthy to ponder how the autoimmune antibodies are produced and activated after CIK cell transfusion. Pieces of evidence have implied that immune response plays an important role in the pathogenesis

of ITP, which involves a complex interaction among antigen-presenting cells (APCs), T helper (Th) cells, regulatory T (Treg) cells and B cells [39,40]. CD4⁺ Th cells may facilitate B-cell activity and autoantibody production. It tends to show an increased Th1/Th2 ratio which always refers to the balance between IL-4 and IFN- γ in untreated ITP patients. CD8⁺ T cells may prevent apoptosis of autologous megakaryocytes and lead to impaired platelet production [41]. Decreased numbers and impaired function of Treg cells, as well as the effects of cytotoxic T lymphocyte (CTL) also contribute to the pathogenesis of ITP [42]. Tregs suppress the production of cytokines by CD4⁺ and CD8⁺ T cells and limit CD8⁺ T-cell cytotoxicity [43]. An increased number of Tregs has been seen in ITP patients undergoing treatment with corticosteroids [44] or rituximab [45], or the combination [46]. Clinical research discovered that CIK cell therapy may increase T cell subgroups CD3⁺ and CD4⁺, decrease CD8⁺ cells, and significantly increase the ratio of CD4⁺/CD8⁺ cells [47]. Moreover, CIK cells may decrease the CD4⁺CD25⁺CD127⁻ Tregs and produce a variety of cytokines, such as IL-2, IL-4, IL-10, IL-13, IL-17, IFN- γ , TNF- α and TGF- β enhancing cytotoxicity and changing the tumor microenvironment in favor of tumor eradication [48]. Increased secretion of these cytokines could be related to activation of macrophages and B cells which is a known feature of ITP [6,49]. Hence, imbalance in the ratio of Th cells, decreased numbers or impaired function of Treg cells and excessive secretion of cytokine induced abnormal activation of B cells, may be the possible reasons for the occurrence of ITP following CIK immunotherapy. However, definitive factors responsible for ITP during and after CIK cell therapy are still elusive.

Table 1. Phenotype analysis of three transfusions of cytokine-induced killer cells.			
Cell control	The first time	The second time	The third time
Total cell count	3.9 × 10 ⁹	4.5 × 10 ⁹	4.1 × 10 ⁹
CD3 ⁺	98.2%	97.5%	98.7%
CD3 ⁺ CD8 ⁺	71.4%	65.5%	80.9%
CD3 ⁺ CD4 ⁺	17.9%	31.0%	9.6%
CD3 ⁺ CD56 ⁺	7.3%	5.2%	8.7%

Table 2. Antiplatelet antibodies detection results.		
Items	Results	Reference values
PAIgG	103.4 ng/10 ⁷ PA	0–108 ng/10 ⁷ PA
PAIgM	55.0 ng/10 ⁷ PA	0–40 ng/10 ⁷ PA
PAIgA	19.6 ng/10 ⁷ PA	0–22 ng/10 ⁷ PA

Table 3. Phenotype analysis of three transfusions of cytokine-induced killer cells.

Cell control	The first time	The second time	The third time
Total cell count	3.9×10^9	4×10^9	3.9×10^9
CD3 ⁺	97.5%	98.1%	98.5%
CD3 ⁺ CD8 ⁺	73.4%	78.5%	69.9%
CD3 ⁺ CD4 ⁺	21.9%	24.7%	30.6%
CD3 ⁺ CD56 ⁺	8.4%	7.6%	10.6%

Table 4. Antiplatelet antibodies detection results.

Items	Results	Reference values
PAIgG	300.0 ng/10 ⁷ PA	0-108 ng/10 ⁷ PA
PAIgM	2.9 ng/10 ⁷ PA	0-40 ng/10 ⁷ PA
PAIgA	40.0 ng/10 ⁷ PA	0-22 ng/10 ⁷ PA

Treatment is rarely required if the platelet count is above $50 \times 10^9/L$. If the platelet count falls below $30 \times 10^9/L$, treatment should be administered based on patient's age, bleeding conditions and other associated medical conditions [5]. Corticosteroids remain the most commonly used first-line therapy for ITP, and more than 80% of patients respond to corticosteroids [5,50]. In our cases, the CIK cell therapy was stopped once they were diagnosed with ITP. One patient received corticosteroids due to an extremely low platelet count and bleeding risk. After which, the patient's platelet count recovered immediately. The other patient discontinued the CIK treatment no further treatment and their platelet count also increased.

In conclusion, it is reported for the first time that CIK cell therapy is associated with ITP. More cases need found and studied to help confirm and understand the relationship, etiology and mechanism between CIK and ITP. Though clinicians should be aware of some potential or unnoticed side effects of CIK cell therapy, such as ITP, this is still quite rare. CIK cell therapy remains a safe and effective method for cancer treatment.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Informed consent disclosure

The authors state that they have obtained verbal and written informed consent from the patient/patients for the inclusion of their medical and treatment history within this case report.

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Executive summary

- Idiopathic thrombocytopenic purpura (ITP) is an autoimmune condition occasionally associated with solid tumor.
- Cytokine-induced killer (CIK) cell therapy is a minor toxic immunotherapy method of cancer treatment as we thought before.
- Our report draws attention to the occurrence of ITP following CIK cell therapy for the first time.
- Imbalance ratio of Th cells, decreased numbers or impaired function of Treg cells and excessive secretion of cytokine induced abnormal activation of B cells, may be the possible reasons.
- More cases need to be studied to confirm the mechanisms between ITP and CIK.

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