Acquired aplastic anemia (AA), characterized by persistent pancytopenia and bone marrow (BM) hypoplasia, is a rare and life-threatening disorder (1). Immune-mediated suppression of hematopoiesis plays a central role in the pathogenesis of AA, which is further confirmed by the favorable response to immunosuppressive treatment (IST) with antithymocyte globulin (ATG) and cyclosporine A (CsA) (2–4). Aberrant immunities such as abnormally polarized T-helper (Th) 1 cells, dysregulated CD8+ cytotoxic T cells, and immoderate secretion of inhibitory hematopoietic cytokines have been widely studied in the past years (5, 6). Recently, decreased numbers and impaired immunosuppressive function of circulating regulatory T cells (Tregs) and immoderate Th17 responses have also been considered to contribute to the pathogenesis of AA (7–9). Although a trigger-related abnormal T-cell response has been postulated as the pathogenetic mechanism of AA, the exact factors responsible for the breakage of immunological self-tolerance in patients with AA remain uncertain.

In addition to its well-recognized role in the regulation of calcium/phosphate metabolism, there was accumulating evidence that 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3], the most biologically active form of vitamin D, was a critical modulator of immune response (10, 11). The immune
modulatory activities of 1,25(OH)\textsubscript{2}D\textsubscript{3} were mediated by binding to the vitamin D receptor (VDR), which was extensively expressed by monocytes and activated lymphocytes (12). 1,25(OH)\textsubscript{2}D\textsubscript{3} appeared to have pleiotropic effects on both the innate and the adaptive immunities. It has been indicated that 1,25(OH)\textsubscript{2}D\textsubscript{3} could inhibit the differentiation and maturation of dendritic cells (DCs) by decreasing the expression of MHC class II and costimulatory molecules (13). Additionally, 1,25(OH)\textsubscript{2}D\textsubscript{3} was a potent inhibitor of T-cell proliferation via blocking the transition from early G\textsubscript{1} phase to late G\textsubscript{1} phase (10, 14). Moreover, 1,25(OH)\textsubscript{2}D\textsubscript{3} suppressed the production of Th1- and Th17-type cytokines, while promoting the secretion of Th2-related cytokines (15, 16). More importantly, 1,25(OH)\textsubscript{2}D\textsubscript{3} induced the expansion of Tregs, further contributing to the maintenance of immune tolerance (17). It has been reported that insufficiency of 1,25(OH)\textsubscript{2}D\textsubscript{3} was associated with the susceptibility and/or disease severity of some autoimmune diseases, such as Crohn’s disease (CD) and rheumatoid arthritis (18).

VDR, a member of the nuclear receptor superfamily, plays an essential role as a mediator of 1,25(OH)\textsubscript{2}D\textsubscript{3} signaling. Upon activation, VDR eventually binds to VDR-response elements to regulate the expression of target genes involved in diverse biological effects (19). The regulation of VDR under basal conditions and upon induction was multifaceted: shaped by environment, genetics, and epigenetics (20). In the past years, many studies have focused on the single nucleotide polymorphisms (SNPs) of VDR gene related to the regulation of gene expression and protein production. Four SNP sites of VDR gene, including FokI, BsmI, TaqI, and ApaI, have been extensively investigated. The ‘B’ allele of BsmI and the ‘t’ allele of TaqI were associated with lower levels of VDR protein, while the TT (C/C) genotype of TaqI showed a trend toward higher levels of VDR protein (21). Additionally, the expression of VDR gene could be upregulated by 1,25(OH)\textsubscript{2}D\textsubscript{3} itself, at the transcription and/or translation level (22, 23). Decreased expression or dysfunction of VDR was involved in diverse autoimmune diseases, such as CD, type I diabetes mellitus, and multiple sclerosis (MS), which made it a strong focal point for exploring the underlying pathogenic mechanism of autoimmune diseases and their possible prevention (20).

To explore the participation of 1,25(OH)\textsubscript{2}D\textsubscript{3} and VDR in the pathogenesis of AA, we measured the plasma levels of 25(OH)D\textsubscript{3}, which served as the primary indicator of vitamin D status in vivo, and then measured the mRNA expression of VDR by real-time PCR. We also evaluated the effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} on the proliferation, differentiation, and cytokines secretion of peripheral blood mononuclear cells (PBMCs) from patients with AA. Our findings suggested that decreased expression of VDR might contribute to the hyperimmune status of patients with AA, and appropriate supplementation of vitamin D derivatives could partly correct the aberrant immunity by strengthening signal transduction through VDR.

**Materials and methods**

**Patients and controls**

Sixty-three patients with AA (median age 31 yr, 33 males and 30 females) and forty-four age-matched healthy controls (median age 33 yr, 23 males and 21 females) were included in this study after approval by the Ethics Committees of the Institute of Hematology, Chinese Academy of Medical Sciences and Peking Union Medical College according to the guidelines of the Declaration of Helsinki (ethics number: KT2014005-EC-1). Written informed consents were obtained from all the patients and controls. The diagnosis and severity classification of AA were established according to the international criteria (24). This cohort of patients included 25 cases with non-severe AA (Non-SAA) and 38 cases with SAA. Among all the patients, 48 were enrolled before receiving any specific therapy and the remaining were in complete response (CR) after IST consisting of ATG and CsA.

**Cell culture and proliferation assays**

PBMCs were separated from heparin-anticoagulated whole-blood samples by Ficoll–Hypaque (1.077 g/mL) density-gradient centrifugation. Parts of isolated PBMCs were collected to measure the mRNA expression of VDR. In some experiments, isolated PBMCs (1 × 10\textsuperscript{6}) were cultured in 1 mL RPMI-1640 medium containing 10% fetal calf serum, 1% glutamine, and 1% penicillin/streptomycin with or without 10\textsuperscript{-7} M 1,25(OH)\textsubscript{2}D\textsubscript{3} (Sigma, St Louis, MO, USA) and meanwhile stimulated with 5 μg/mL phytohaemagglutinin (Sigma) and 5 ng/mL recombinant interleukin (IL)-2 (Peprotech, Rocky Hill, NJ, USA) for 72 h at 37°C in 5% CO\textsubscript{2}. After incubation, the culture supernatants were collected for the detection of cytokines, and the cells were harvested for the extraction of total RNA.

Cell proliferation assays were determined using Cell Counting Kit-8 (CCK8) method. As a WST-8 tetrazolium reagent, CCK8 is metabolized by mitochondrial dehydrogenase of viable cells to a colorimetric dye and the cell numbers are proportional to the absorbance intensity at 450 nm. Based on these, we used this assay to evaluate lymphocytes proliferation. Briefly, cells were plated in flat-bottomed 96-well microplates at 2 × 10\textsuperscript{5}well and cultured as described above (six duplication wells per group). CCK8 (20 μL/well) (Dojindo, Kumamoto, Japan) was added during the last 4 h of culture. After incubation, the absorbance at 450 nm was detected using a microplate reader. The percentage of inhibition was calculated using the following formula: 1 – (absorbance of treated cells/absorbance of control cells) × 100.
Flow cytometry

After incubation with or without 1,25(OH)2D3 for 48 h as described above, we restimulated cultured PBMCs with 50 ng/mL phorbol 12-myristate 13-acetate and 1 μg/mL ionomycin (Sigma) in the presence of 3 μg/mL brefeldin A (eBioscience, San Diego, CA, USA) for 5 h at 37°C in 5% CO2. Cells were then labeled with FITC-conjugated anti-CD3 and APC-conjugated anti-CD8 antibodies (Biolegend, San Diego, CA, USA) for 20 min at room temperature. Subsequently, cells were fixed by fixation buffer (Biolegend), followed by permeabilization with permeabilization wash buffer (Biolegend). Cells were then stained with PE-conjugated antibodies of IL-17 and IL-4, PE-Cy7-conjugated anti-interferon-γ (IFN-γ), and isotype antibodies (all from Biolegend) for 30 min at room temperature, washed twice, and resuspended in 1% paraformaldehyde for subsequent cytometric analysis.

Quantification of gene expression by real-time PCR

Total RNA of freshly isolated or cultured PBMCs was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The reverse transcription reactions were carried out using the TransScript First-Strand cDNA Synthesis Supermix (TransGen Biotech, Beijing, China) according to the manufacturer’s instructions. Real-time PCR was performed with 2× SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) using the Applied Biosystems Gene Amp 7500 Sequence Detection System. The primer sequences were listed in supplemental Table 1. The PCR conditions were as follows: 40 cycles of three steps (94°C for 15 s, 60°C for 1 min, 72°C for 30 s) after initial denaturation (94°C for 10 min). The relative quantification (RQ) of target mRNA expression was calculated relative to the expression of β-actin using the 2^ΔΔCt method.

Enzyme-linked immunosorbent assay (ELISA)

Plasma and culture supernatants were stored at −80°C until the ELISA was performed. Plasma 25(OH)D3 (Immunodiagnostic Systems Limited, Gaithersburg, MD, USA) and the concentrations of IFN-γ, tumor necrosis factor-α (TNF-α), IL-17A, IL-10, and transforming growth factor-β1 (TGF-β1) in culture supernatants (NeoBioscience Technology, Shenzhen, China) were measured by ELISA kits, all according to the manufacturer’s instructions. The limits of detection were 5 nmol/L for 25(OH)D3; 8 pg/mL for IFN-γ, TNF-α, and IL-17A; 1 pg/mL for IL-10; and 15 pg/mL for TGF-β1.

Statistical analysis

All analyses were performed using SPSS version 16.0 software (SPSS, Chicago, IL, USA). The results were presented as mean ± SEM. Plasma 25(OH)D3 levels between patients with AA and healthy controls were analyzed using one-way ANOVA. The correlations between plasma 25(OH)D3 levels and clinical parameters were determined by the Spearman correlation test. Other statistical differences were evaluated by nonparametric Mann–Whitney U-test between unpaired data and by Wilcoxon signed-rank test for two related samples. A P value < 0.05 was considered statistically significant.

Results

Correlations between plasma 25(OH)D3 levels and clinical parameters of patients with AA

We first detected the plasma 25(OH)D3 levels in patients with AA and healthy controls; however, no statistical difference was found among untreated patients with AA, patients in CR, and healthy controls (37.7 ± 1.7, 43.4 ± 2.4, and 36.8 ± 2.6 nmol/L, respectively). A similar result was observed when we further stratified according to the disease severity (Non-SAA and SAA) (Fig. 1A). We next analyzed the correlations between plasma 25(OH)D3 levels and clinical parameters of patients with AA, and we found that plasma 25(OH)D3 levels showed significantly positive correlations with platelet counts (r = 0.40, P = 0.02; Fig. 1B) and the proportion of CD3+CD16/CD56+ natural killer T (NKT) cells (r = 0.44, P = 0.009; Fig. 1C), while plasma 25(OH)D3 levels were inversely correlated with the percentage of CD19+ B cells (r = −0.36, P = 0.04; Fig. 1D) in patients with AA.

Decreased mRNA expression of VDR in PBMCs from untreated patients with AA

We next measured the mRNA expression of VDR in PBMCs from untreated patients with AA and healthy controls by real-time PCR. As illustrated in Fig. 2, the VDR mRNA level relative to β-actin was significantly lower in untreated patients with AA than that of healthy controls (P < 0.0001), suggesting the 1,25(OH)2D3 signaling through VDR might be abnormally weakened in de novo patients with AA.

Effects of 1,25(OH)2D3 on the proliferation of PBMCs

Because of the decreased expression of VDR in patients with AA, we evaluated whether 1,25(OH)2D3 exerted any effects on the proliferative capacity of PBMCs from patients with AA. Firstly, we stimulated PBMCs with 1,25(OH)2D3 (10−11, 10−10, 10−9, 10−8, 10−7 M) or ethanol vehicle alone for 72 h, and then detected the cellular proliferation using CCK8 method. 1,25(OH)2D3 stimulation affected the proliferation of PBMCs in a dose-dependent manner, while
ethanol exerted no effect (data not shown). So we chose 10⁻⁷ M as the appropriate concentration for subsequent experiments. As shown in Fig. 3A, 1,25(OH)₂D₃ significantly decreased the proliferation of PBMCs from patients with AA (P = 0.01) and healthy controls (P = 0.008). Interestingly, the percentage of inhibition was lower in patients with AA than in healthy controls (14.6 ± 2.5% vs. 22.5 ± 1.9%, P = 0.03; Fig. 3B), which could be partly explained by the decreased expression of VDR in patients with AA.

Effects of 1,25(OH)₂D₃ on the secretion of IFN-γ, TNF-α, IL-17A, IL-10, and TGF-β1 in the supernatants of PBMCs

To evaluate the effects of 1,25(OH)₂D₃ on the secretion of representative cytokines in culture supernatants, we detected the levels of IFN-γ, TNF-α, IL-17A, IL-10, and TGF-β1 by ELISA. We firstly compared the levels of these cytokines in the supernatants of PBMCs not incubated with 1,25(OH)₂D₃ between patients with AA and healthy controls. The levels of IFN-γ and TNF-α in the supernatants of cultured PBMCs from patients with AA were significantly higher than those of healthy controls (P = 0.001 and P < 0.0001, respectively). Additionally, the levels of IL-17A, IL-10 and TGF-β1 were markedly lower in patients with AA than those of healthy controls (P = 0.02, P = 0.005 and P < 0.001, respectively).

We determined the effects of 1,25(OH)₂D₃ on the secretion of these cytokines. 1,25(OH)₂D₃ suppressed the production of IFN-γ, TNF-α, and IL-17A but promoted the secretion of TGF-β1 in the culture supernatants of PBMCs from both patients with AA and healthy controls (P < 0.01, Fig. 3C–E, G). The expression of IL-10 remained to be unchanged either in patients with AA or in healthy controls (Fig. 3F).

Effects of 1,25(OH)₂D₃ on the differentiation of Th17 cells of PBMCs

To confirm the effects of 1,25(OH)₂D₃ on the differentiation of Th17 cells, we detected the proportion of Th17 cells by flow cytometry. After 1,25(OH)₂D₃ treatment, the percentage of Th17 cells significantly decreased in both patients with AA and healthy controls (P = 0.008 and P = 0.007, respectively; Fig. 4A).

Effects of 1,25(OH)₂D₃ on the Th1/Th2 and Tc1/Tc2 differentiation of PBMCs

To verify the effects of 1,25(OH)₂D₃ on the differentiation of type 1 and type 2 T cells, we measured the proportions of Th1, Tc1, Th2, and Tc2 subsets of T lymphocytes with or without 1,25(OH)₂D₃ stimulation. 1,25(OH)₂D₃
significantly decreased the percentages of Th1 and Tc1 cells, but increased the proportions of Th2 and Tc2 cells in cultured PBMCs from patients with AA and healthy controls, accompanied with the sharply decreased ratios of Th1/Th2 and Tc1/Tc2 ($P < 0.05$, Fig. 4B–I). Interestingly, the decreased ratio of Th1 cells in patients with AA was notably greater than healthy controls.

**Effects of 1,25(OH)$_2$D$_3$ on the mRNA expression of specific transcription factors T-bet, GATA3, ROR$\gamma$t, and Foxp3 of PBMCs**

We further evaluated the effects of 1,25(OH)$_2$D$_3$ on the mRNA expression of T-bet, GATA3, ROR$\gamma$t, and Foxp3 by real-time PCR. As expected, 1,25(OH)$_2$D$_3$ stimulation led to decreased expression of ROR$\gamma$t but increased expression of GATA3 and Foxp3 in patients with AA and healthy controls ($P < 0.05$). Surprisingly, 1,25(OH)$_2$D$_3$ decreased the mRNA expression of T-bet in patients with AA, but had no effect on healthy controls. Combined with the sharp decrease of Th1 cells in patients with AA after 1,25(OH)$_2$D$_3$ treatment, we speculated that 1,25(OH)$_2$D$_3$ might regulate the Th1-type immune response in different ways between patients with AA and healthy controls. Moreover, 1,25(OH)$_2$D$_3$ sharply decreased the ratio of T-bet/GATA3 in both patients with AA and healthy controls ($P = 0.002$; Fig. 5).

**Effects of 1,25(OH)$_2$D$_3$ on the mRNA expression of VDR of PBMCs**

As 1,25(OH)$_2$D$_3$ itself is able to regulate the expression of VDR gene, we next determined whether the mRNA
expression of VDR in cultured PBMCs was influenced by 1,25(OH)2D3 treatment. 1,25(OH)2D3 markedly promoted the mRNA expression of VDR in healthy controls (P = 0.003). Although the mRNA expression of VDR also elevated in patients with AA, no statistical difference was found (P = 0.64; Fig. 6).

**Discussion**

Deficiency in vitamin D or VDR has been implicated in a range of autoimmune diseases. This was the first study to explore the potential role of vitamin D/VDR in the pathogenesis of AA. In the current study, we showed that the plasma 25(OH)D3 levels were comparable between patients with AA and healthy controls. Further analysis indicated that the plasma 25(OH)D3 levels correlated positively with platelet counts and the percentage of NKT cells, but negatively with the percentage of B cells in patients with AA. It has been reported that vitamin D inhibited the generation of memory B cells from naïve B cells, suppressed B cells proliferation, induced B cells apoptosis, and maintained the development and function of NKT cells (25, 26), which might explain the close correlations between plasma 25(OH)D3 levels and the percentages of NKT cells and B cells in our study. As NKT cells disproportionally decreased in patients with AA and this deficiency played a role in immune dysregulation (27), vitamin D might correct the abnormal immunity of AA partly by increasing the number of NKT cells.

The biological activities of 1,25(OH)2D3 are mediated by binding to VDR, which serves as a transcription factor to modulate the expression of numerous target genes. Besides its critical roles in calcium homeostasis, ligand-bound VDR plays an important role in the maintenance of immune tolerance. Hindered expression and function of VDR have been implicated to be involved in the pathophysiology of diverse diseases, including cancer, MS, and tuberculosis (20). We found that the mRNA expression of VDR was significantly decreased in untreated patients with AA than in healthy controls, suggesting the vitamin D-VDR pathway might be
weakened in patients with AA, thus contributing to the pathogenesis of AA. A limitation of our study was that the protein levels of VDR were not determined because of the limited counts of PBMCs. However, previous studies have demonstrated that the mRNA and protein levels of VDR in various tissues, such as placental endothelium, squamous epithelium, and parathyroid cells, exhibited similar trends without in vivo or in vitro intervention (28–30). Further studies are needed to validate the concordant results between the mRNA and protein levels of VDR in patients with AA.

Given the fact that the expression of VDR gene could be upregulated by vitamin D itself, we hypothesized that appropriate vitamin D supplementation could partially reverse the hyperimmune status of AA by strengthening the signal transduction through VDR directly or indirectly. We herein further investigated the effects of 1,25(OH)2D3 on the T-cell response of patients with AA to evaluate the potential involvement of 1,25(OH)2D3-VDR pathway in the pathogenesis of AA.

Consistent with previous study (31), 1,25(OH)2D3 inhibited mitogen-induced T lymphocyte proliferation in patients with AA and healthy controls in a dose-dependent manner. Most likely, this antiproliferation effect of 1,25(OH)2D3 appeared to be mediated, at least in part, by inhibition of IL-2 production (32). Interestingly, 1,25(OH)2D3 was less efficient in suppressing the proliferative responses of PBMCs from patients with AA than those from healthy controls, which could be explained by the weakened signal transduction attributed to decreased VDR and/or the hyperreactive responses of effector T cells in patients with AA.

Immune-mediated destruction of hematopoietic progenitor/stem cells is the major cause of AA in most patients. It has been well established that an abnormal type 1 cell-dominant immune response developed in AA, leading to immoderate secretion of proinflammatory cytokines, such as IFN-γ, IL-2, and TNF-α, which exhibited synergistic effects on the suppression of hematopoiesis in patients with AA (33–35). In
In accordance with previous studies, we also showed significantly elevated levels of IFN-γ and TNF-α and decreased levels of Th2-related cytokine IL-10 in the supernatants of cultured PBMCs from patients with AA. 1,25(OH)2D3 promoted T cell shifts from Th1 to Th2 (36), which might help limit the potential BM damage associated with overactivation of type 1 cell immune response in AA. Supportive of this, our findings showed that 1,25(OH)2D3 inhibited the differentiation of Th1/Tc1 cells, mRNA expression of T-bet, and secretion of IFN-γ and TNF-α, but promoted the differentiation of Th2/Tc2 cells and mRNA expression of GATA3 in patients with AA. Interestingly, the expression of T-bet remained to be unchanged after 1,25(OH)2D3 treatment in healthy controls. As it has been reported that 1,25(OH)2D3 could inhibit Th1 response directly by binding to vitamin D response elements on the promoter of IFN-γ gene, or indirectly by suppressing the differentiation of monocytes into antigen-presenting dendritic cells (APCs) (13, 37), we speculated that the regulatory mechanism of type 1 cell immune responses mediated by 1,25(OH)2D3 might be partially different between patients with AA and healthy controls, which needs further studies to validate.

Th17 cells have been reported to contribute to the pathophysics of AA, particularly in the early stage (8). We showed that the percentage of Th17 cells and IL-17A levels of cultured PBMCs from patients with AA and healthy controls were significantly decreased after incubation with 1,25(OH)2D3, accompanied with the decreased mRNA expression of RORγt. Therefore, the elevated Th17 immune responses in patients with AA might be regulated by vitamin D supplementation. Interestingly, the IL-17A levels in cultured supernatants of PBMCs from patients with AA were lower than those in healthy controls. We speculated that the abnormality of Th17 cells in AA might make them less likely to survive and differentiate than their healthy counterparts under this specific stimulation condition in vitro, thus leading to the decreased secretion of IL-17A.

Tregs play a central role in the maintenance of immune homeostasis (38, 39). Our previous study has established that both the frequency and immunosuppressive function of Tregs were impaired in patients with AA (9). 1,25(OH)2D3 could induce and stimulate Tregs through an indirect pathway, via APCs and DCs, or through a direct pathway, via an endocrine effect or the intracrine conversion of 25(OH)D to 1,25(OH)2D3 by Tregs themselves (40). We disclosed the markedly elevated Foxp3 mRNA after 1,25(OH)2D3 treatment. Moreover, 1,25(OH)2D3 promoted the secretion of TGF-β1 but not IL-10 in the culture supernatants of PBMCs from patients with AA. As IL-10 was extensively produced by several cell types, suppression of other IL-10-producing cells mediated by 1,25(OH)2D3 might contribute to the unchanged levels of IL-10. Collectively, our data suggested that 1,25(OH)2D3 could reverse the aberrant immune response and favor the development of immune tolerance in patients with AA by skewing T-cell differentiation toward Th2/Tc2 or Tregs phenotype, even though the decreased expression of VDR in AA.

We also evaluated the effects of 1,25(OH)2D3 on the expression of VDR gene. The significantly increased mRNA expression of VDR after 1,25(OH)2D3 treatment was observed in healthy controls but not in patients with AA, suggesting the expression of VDR under basal conditions and upon induction was both defective in patients with AA. As VDR protein was also known to be regulated post-transcriptionally upon 1,25(OH)2D3 intervention (41), further studies are warranted to detect the expression levels of VDR protein. The expression and function of VDR were tightly regulated by three main factors, namely environment, genetics, and epigenetics (20). Accumulating evidence has indicated that SNPs of VDR gene had profound effects on the expression and function of VDR and were associated with the susceptibility of some autoimmune diseases. Therefore, further studies are needed to clarify whether SNPs of VDR gene are related to the decreased expression of VDR in AA, which may facilitate our better understanding of the pathogenesis of AA.

Due to the pleiotropic effects of 1,25(OH)2D3 on immune modulation, much attention has been focused on its immunotherapeutic potential in autoimmune diseases. A trial of vitamin D supplementation was shown to reduce disease activity scores in a small cohort of patients with CD, suggesting the therapeutic benefits from elevating vitamin D levels (42). Imai et al. reported that patients with AA and myelodysplastic syndrome were successfully treated with an intermittent administration of high-dose 1(OH)D3, an active analog of 1,25(OH)2D3, and the only adverse effect was hypercalcemia, which was controllable by decreasing the dose (43). Additionally, it has been reported that EB1089, an analog of vitamin D, promoted the proliferation and osteogenic differentiation of BM mesenchymal stem cells (MSCs) from patients with systemic lupus erythematosus by Smad 1/5/8 signaling pathway (44). Given the fact that BM-MSCs from patients with AA were also defective in proliferation, osteogenic differentiation, and hematopoiesis support (6), 1,25(OH)2D3 might ameliorate the symptoms of AA by modulating both the immune cells and BM-MSCs. As supplementation of appropriate vitamin D is generally safe, practical, and inexpensive, application of 1,25(OH)2D3, particularly of its non-calcemic analogs, may be a promising adjunctive therapy to conventional ATG- and CsA-based IST for the treatment of AA.

In summary, the present study identified the significantly decreased mRNA expression of VDR in patients with AA. Further in vitro experiments indicated that 1,25(OH)2D3 exerted great effects in both the differentiation of T cells and the secretion of anti/pro-inflammatory cytokines in patients with AA. All these data suggest that decreased expression of VDR may be responsible for the breakdown.
of immune tolerance in AA, and appropriate vitamin D supplementation may help reverse the abnormal immunity of AA by enhancing signal transduction through VDR. Randomized clinical trials are needed to evaluate the effectiveness of vitamin D treatment in AA and help guide therapeutic decisions in the future.

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Authorship contributions

W Yu, ML Ge, and YZ Zheng designed the research study; W Yu, ML Ge, SH Lu, J Shi, SZ Feng, and XX Li performed the research and analyzed the data; JZ Zhang, M Wang, JB Huang, YQ Shao, ZD Huang, J Zhang, and N Nie contributed sample collection and essential reagents or tools; and W Yu and YZ Zheng wrote the manuscript.

Conflict of interest and sources of funding

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44. Yu et al. Supporting Information
Additional Supporting Information may be found in the online version of this article:
Table S1. Primer sequences for real-time PCR.