Anti-interleukin-5-neutralizing antibody attenuates cardiac injury and cardiac dysfunction by aggravating the inflammatory response in doxorubicin-treated mice

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Abstract

Previous studies have demonstrated that interleukins (ILs) are closely associated with doxorubicin (DOX)-induced cardiac injury. IL-5 is an important member of the IL family, and this study was performed to investigate whether IL-5 affects DOX-induced cardiac injury and its underlying mechanisms. The cardiac IL-5 expression was first detected and the results showed that cardiac IL-5 levels were significantly lower in DOX-treated mice, and IL-5 was mainly derived from cardiac macrophage (Mø). In addition, some DOX-treated mice received an injection of anti-IL-5-neutralizing antibody (nAb), and we found that treatment with a mouse anti-IL-5 nAb significantly upregulated the levels of myocardial injury markers, aggravated cardiac dysfunction, increased M1 macrophage (Mø1) and decreased M2 macrophage (Mø2) differentiation, and promoted apoptotic marker expression. Furthermore, the effect of mouse IL-5 nAb on DOX-induced Mø differentiation and its role on mouse cardiac myocyte (MCM) cells apoptosis were detected in vitro, and the results exhibited that mouse IL-5 nAb promoted Mø1 differentiation but inhibited Mø2 differentiation in vitro and alleviated apoptosis in MCM cells. Our results found a mouse anti-IL-5 nAb-aggravated DOX-induced cardiac injury and dysfunction by alleviating the inflammatory response and myocardial cell apoptosis.

Keywords: anti-IL-5-neutralizing antibody; doxorubicin; inflammatory response; macrophage differentiation; myocardial cell apoptosis

Introduction

Doxorubicin (DOX) is one of the most popular chemotherapy drugs and could be used in the clinical treatment of a variety of cancers, such as leukemia, carcinoma, and soft tissue sarcoma (Duggan and Keating, 2011). However, DOX can cause a variety of serious clinical complications, especially cardiac injury, which can increase the risk of death (Octavia et al., 2012). Although the specific mechanisms remain unknown, numerous studies have demonstrated that the cardiac inflammatory response and myocardial apoptosis are important factors underlying the progression of DOX-induced cardiac injury (Yuan et al., 2018).

Interleukin-5 (IL-5) is an important member of the IL-2 superfamily, and mainly secreted by immune cells such as macrophages (Mø) and lymphocytes (especially the CD4+ T lymphocytes), but less secreted by smooth muscle cells and endothelial cells (Sewell et al., 1998; Deo et al., 2010; Takatsu, 2011; Park et al., 2017). IL-5 binds to IL-5 receptor (IL-5R) on target cells, activates the Janus Kinase (JAK) 1/2 and signal transducer and activator of transcription 1/5

Abbreviations: ANOVA, analysis of variance; Arg-1, arginase-1; CK-MB, creatine kinase myocardial-bound; DOX, doxorubicin; ELISA, enzyme-linked immunosorbent assay; HE, hematoxylin and eosin; HR, heart rate; IFN-γ, interferon γ; ILs, interleukins; IL-5R, IL-5 receptors; iNOS, inducible nitric oxide synthase; JAK1/2, Janus Kinase 1/2; LDH, lactate dehydrogenase; LV, left ventricle; LVEF, left ventricle ejection fraction; LVEDP, left ventricular end-diastolic pressure; LVFS, left ventricle fractional shortening; LVSP, left ventricular systolic pressure; Mø, macrophage; MCMs, mouse cardiac myocytes; Mø1, M1 macrophage; Mø2, M2 macrophage; nAb, neutralizing antibody; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; STAT1/5, signal transducer and activator of transcription 1/5; SMCs, smooth muscle cells; SD, standard deviation; TNF-α, tumor necrosis factor α; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; WT, wild-type; +dP/dt max, maximal slope of the systolic pressure increment; −dP/dt max, maximal slope of the diastolic pressure decrement

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(STAT1/5) signaling pathway and initiates downstream signals (Takaki et al., 1994; Kouro et al., 1996; Ogata et al., 1998). Previous studies have indicated that IL-5 participates in a variety of diseases by regulating immune effects and the inflammatory response. In a mouse model, treatment with an anti-IL-5-neutralizing antibody (nAb) before antigen inhalation suppressed airway hyperreactivity (Nakajima et al., 1992; Mauser et al., 1995). In angiotensin II-infused apolipoprotein E knockout mice, valsartan treatment increased Th2 cell expression and IL-5 levels and decreased the area of atherosclerotic plaques, while mouse anti-IL-5 nAb signaling reversed these effects (Meng et al., 2015).

IL-5 has also been demonstrated to be closely related to other cardiovascular diseases. In a recent study, anti-IL-5 nAb treatment increased ejection fraction and stabilized cardiac function (Song et al., 2017). Another study reported that IL-5 was an important protective factor in cardiovascular disease, while individuals with IL-5 gene mutations were more likely to suffer from cardiovascular diseases (McLeod et al., 2016). IL-5 was reported to alleviate atherosclerosis in both apolipoprotein E knockout and LDL receptor-deficient mice (Meng et al., 2015; Zhao et al., 2015). However, the role of IL-5 in DOX-induced cardiac injury remains unknown. In this study, a mouse anti-IL-5 antibody was used to explore the effects of IL-5 on DOX-induced cardiac injury and explore its possible mechanism.

### Materials and methods

#### Animals and their models

Male wild-type (WT) mice with a C57BL/6 background purchased from HFK Bioscience were used in this study. First, the mice received intraperitoneal (i.p.) injections of DOX (purity ≥ 98%; Novopharm, 15 mg/kg) for 5 days, while an equal volume of saline was administered to mice in the control group (N = 8 for each group). In addition, the mice received phosphate-buffered saline (PBS) (50 µL), IL-5 nAb (50 µg), or an equivalent amount of isotype immunoglobulin G (Meng et al., 2015). Beginning 1 day later, all the mice were treated with DOX injections for 5 days, and some of the mice received PBS and were treated with saline as a control (N = 10 for each group). These mice were observed every 12 h and weighed every 24 h. Bodyweight was measured before the study and on the fifth day of DOX treatment. Heart weight was also detected after the mice were euthanized. This study was approved by the Institutional Animal Care and Use Committee of the renmin hospital of wuhan university.

#### Western blot analysis

Primary myocardial cells were lysed, and total protein was collected. Total protein was then extracted and detected with a BCA Protein Assay Kit (Thermo Fisher Scientific). Approximately 25 µg of protein was separated by electrophoresis on Laemmli sodium dodecyl sulfate (SDS) polyacrylamide gels. After electrophoresis, the samples were transferred to Immobilon-FL Polyvinylidene fluoride membranes (Millipore). The membranes were blocked with 5% nonfat milk and then incubated with anti-IL-5, anti-Bax, anti-Bcl2, anti-cleaved-caspase3 (Cle-cas3), and anti-GADPH antibodies (all from Cell Signaling Technology) at 4°C overnight. The secondary antibodies were incubated at room temperature for 1 h. The blots were scanned using a two-colored infrared imaging system (Odyssey).

#### Histological analysis

After the mice were euthanized under anesthesia, their hearts were isolated and immediately arrested in diastole with 10% KCl. After they were fixed in 4% neutral paraformaldehyde for 5 days, the hearts were embedded in paraffin, cut into 4–5-mm-thick slices and mounted onto slides. Hematoxylin and eosin (H&E) staining was used for histopathological analysis. Anti-CD80 and anti-CD206 antibodies (both from R&D Systems) were used to perform immunohistochemistry staining to label cardiac CD80- and CD206-positive cells, respectively. Anti-IL-5, anti-inducible nitric oxide synthase (iNOS) (Abcam), anti-arginase-1 (Arg-1) (Abcam), and anti-cleaved-caspase3 (Cle-cas3) antibodies were used for immunofluorescence staining to investigate the expression of the cardiac IL-5, iNOS, Arg-1, and Cle-cas3 proteins, respectively. Double immunofluorescence staining was performed using anti-IL-5 and anti-F4/80 antibodies to label the source of IL-5 in cardiac Mø. In addition, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) was performed using a commercially available kit according to the manufacturer's instructions (Millipore) to detect apoptosis.

#### Echocardiography and hemodynamics

Mice were anesthetized with 2% isoflurane, and echocardiography and hemodynamic analyses were performed. In the echocardiography analysis, a MyLab 30CV ultrasound (Esaote SpA) system with a 10-MHz linear array ultrasound transducer was used to detect and record M mode images of the left ventricle (LV) at the papillary muscle level. Then, the heart rate (HR) and LV function, including the LV ejection fraction (LVEF) and fractional shortening (FS), were measured. To perform the hemodynamics analysis, a microtip catheter transducer (Millar, Inc.) was inserted into
the right carotid artery and advanced into the LV; the signals were continuously recorded using a Millar Pressure-Volume system (Millar, Inc.), and the maximal slope of the systolic pressure increment (+dP/dt max) and diastolic pressure decrement (−dP/dt max), left ventricular systolic pressure (LVSP), and left ventricular end-diastolic pressure (LVEDP) were recorded on a beat-by-beat basis.

**Measurement of enzymes and cytokines**

The LV samples were lysed, and the supernatant of each sample was collected. Lactate dehydrogenase (LDH) activity and creatine kinase myocardial-bound (CK-MB) levels were measured in both the serum and the supernatant according to the manufacturer’s instructions (all from Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

**Quantitative reverse-transcription polymerase chain reaction (RT-qPCR)**

The LV samples, Mø, and smooth muscle cells (SMCs) were lysed with TRizol reagent, and total RNA was collected. Approximately 2 µg of RNA was used to synthesize complementary DNA using a reverse transcription kit according to the manufacturer’s instructions. PCR amplification was performed using a LightCycler 480 SYBR Green Master Mix (Roche, Mannheim, Germany). The messenger RNA (mRNA) levels of CD80, CD206, iNOS, Arg1, IL-1β, IL-4, IL-6, IL-10, IL-17, tumor necrosis factor α (TNF-α), interferon γ (IFN-γ), Bax, Bcl2, and caspase3 were measured and normalized to the level of glyceraldehyde 3-phosphate dehydrogenase. The primer sequences used for RT-qPCR are shown in Table 1.

**Cell culture experiment**

Bone marrow-derived Mø were obtained from C57BL/6 mice and prepared as previously described (Lake et al., 1994; Ye et al., 2019). The Mø were cultured in RPMI-1640 with 10% fetal bovine serum (both from Gibco) in an environment with 5% CO2 at 37°C and then treated with saline and DOX (1 µM). In addition, PBS or recombinant mouse anti-IL-5 nAb (50 ng/mL; PeproTech) was added to the medium (Yin et al., 2011). After treatment for 12 h, total mRNA was collected from the Mø for mRNA analysis, and the supernatant of the culture medium was obtained for cytokine detection. Mouse cardiomyocytes (MCMs, purchased from the ATCC) cells were cultured in complete Dulbecco’s modified Eagle’s medium and treated with DOX; the medium described above was then added. The MCM cells were then collected to detect the protein levels of Bax, Bcl2, and Cle-cas3.

**Enzyme-linked immunosorbent assay (ELISA)**

Mouse IL-5 (R&D Systems) levels were measured in serum, and mouse IL-1β, IL-4, IL-6, IL-10, IL-13, IL-17, TNF-α, and IFN-γ (eight above from eBioscience) levels were measured in the supernatant of the culture medium using ELISA kits according to the manufacturer’s instructions. All samples were measured in duplicate.

**Statistical analysis**

All data were analyzed by GraphPad 6 and are expressed as the mean ± standard deviation (SD). Differences between the means of two groups were compared with Student’s t test. Differences between the means of multiple groups were compared with one-way analysis of variance

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**Table 1** Reverse-transcription polymerase chain reaction (RT-PCR) primers used.

| Gene   | Forward primer      | Reverse primer      |
|--------|---------------------|---------------------|
| CD80   | CCATGTCAGAACGGCTATTCT | TCCCCAGCAATGACAGAGCAG |
| nNOS   | TGACCAGCTGAAACTGACTGA | CAGTATGGCACCCGAGCATG |
| IL-1β  | GGGCCTCAAAAGGAAAGATT | TACCAAGGTGGAAAACCTGAC |
| IL-6   | AGTTGCCCTTTGGAGAATGA | TCCACGGTTCCCAGAGAAC |
| IL-17  | TCCAGAAGCTCCAGACTCA | AGCATCTTCGTGAGCCCTGA |
| TNF-α  | CCCAGGACCTTCTCTAATC | ATGGGGCTACGGGTTGCACT |
| IFN-γ  | ACTGGCAAAAGGATGTAAGG | TGAACGTCATTGGAATGCTTGG |
| CD206  | GCAAGGGATGATTTCTTGA | TCTTTTAATGAGCCTGG |
| Arg1   | TGCTATGGGAGAGATGTCT | TTTTGGATGAGCAGGCTTTT |
| IL-4   | ACCAGGTCACAGGGAGAGAGGA | AGGCCCTACAGGAGGCTACCT |
| IL-10  | ATAACGAGCAGCCACTCCCA | GGGCATTTACCTACAGG |
| IL-13  | CAGCAAGGCCCACTCAC | TGGCGAAAGCATGAGGCTT |
| Bax    | TTTCTGATGGAACATCTCA | GATCGATCGGGCACCTTTAG |
| Bcl2   | CAAAGAGATGTGGCTTCTT | CTTTCTGATTTTTTATGAGG |
| GAPDH  | AACCTGTGGACTCTGGAGG | CACATGGGGGTAGGAAAC |

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(ANOVA) followed by Tukey's multiple comparison test. A value of $P < 0.05$ was considered significant.

**Results**

DOX treatment decreased cardiac IL-5 expression

Both western blot and histological analyses were used to measure cardiac IL-5 expression, and the results showed that DOX treatment significantly decreased cardiac IL-5 levels (Figures 1A and 1B). In addition, DOX treatment did not affect IL-5 mRNA levels in T lymphocytes but reduced IL-5 mRNA levels in Mø (Figure 1C). Double staining with anti-IL-5 and anti-F4/80 antibodies also showed that cardiac Mø-derived IL-5 levels were reduced by DOX (Figure 1D).

Anti-IL-5 nAb aggravates DOX-induced cardiac injury

There was no difference in body weight at baseline among the four groups. Treatment with DOX for 5 days significantly reduced body weight, which was further reduced by anti-IL-5 nAb (Figure 2A). Heart weight was reduced in the DOX group and further reduced in the DOX + nAb group (Figure 2B). In addition, the number of vacuolated cardiomyocytes was increased in DOX-treated mice and further increased by anti-IL-5 nAb (Figure 2C). Furthermore, cardiac injury markers, including LDH and CK-MB, were measured in both serum and the heart, in which they showed a trend similar to that observed for the number of vacuolated cardiomyocytes (Figures 2D–F).

Anti-IL-5 nAb exacerbates DOX-induced cardiac injury

Echocardiography showed that the HR, LVEF, and FS were significantly lower in the DOX group than in the control group after 5 days of DOX treatment and that anti-IL-5 nAb exacerbated the decreases observed in these parameters in DOX-treated mice (Figures 3A–C). Similar results were obtained when invasive hemodynamic measurements were obtained, and the anti-IL-5 antibody aggravated DOX-induced LV systolic and diastolic dysfunction (Figures 3D–G).

Anti-IL-5 nAb enhances the DOX-induced inflammatory response

Both intracellular markers (iNOS and Arg-1) and surface markers (CD80 and CD206) of M1 and M2 Mø were detected by histological analysis. The results of surface staining showed that treatment with DOX increased the levels of heart iNOS and that treatment with anti-IL-5 nAb

![Figure 1](image_url)

**Figure 1** Effect of doxorubicin (DOX) treatment for 5 days on cardiac interleukin-5 (IL-5) expression. (A) IL-5 levels were measured in saline and DOX-treated mice by western blot analysis. (B) Immunofluorescence analysis of IL-5 protein expression in the heart in each group (×200). (C) Effect of DOX treatment on IL-5 messenger RNA expression in T lymphocytes and macrophage. (D) Double immunofluorescence staining of anti-F4/80 and anti-IL-5 in saline-treated mice and DOX-treated mice (×200). N=4 in each group. **$P<0.1$ versus the saline group. DAPI, 4',6-diamidino-2-phenylindole; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
Figure 2 Effect of anti-interleukin-5 (IL-5) neutralizing antibody (nAb) on doxorubicin (DOX)-induced cardiac injury. (A) Bodyweight was measured in each group before and 5 days after DOX treatment; \( N = 10 \) in each group. (B) Heart weight was measured at the end of the fifth day in the four groups; \( N = 8 \) in each group. (C) Heart sections were obtained and stained with hematoxylin and eosin. The vacuolated cardiomyocytes were quantified (×200); \( N = 5 \) in each group. (D and E). Serum lactate dehydrogenase (LDH) and creatine kinase myocardial-bound (CK-MB) levels were assessed; \( N = 8 \) in each group. (f) Heart LDH and CK-MB levels were detected; \( N = 4 \) in each group. *\( P < 0.5 \) versus the control group and #\( P < 0.5 \) versus the DOX group. IgG, immunoglobulin G.

Figure 3 Evaluation of left ventricle (LV) function by echocardiography and hemodynamic data in each group. (A–C) Heart rate (HR), left ventricle ejection fraction (LVEF), and fractional shortening (FS) were evaluated by echocardiography in the four groups. (D–G) Positive and negative dP/dt max, left ventricular systolic pressure (LVSP), and left ventricular end-diastolic pressure (LVEDP) were assessed by hemodynamic data in the four groups; \( N = 8 \) in each group. *\( P < 0.5 \) versus the control group and #\( P < 0.5 \) versus the doxorubicin (DOX) group. IgG, immunoglobulin G.
further increased iNOS levels in DOX-treated mice (Figure 4A). Moreover, DOX-induced a decrease in heart Arg-1 expression, and anti-IL-5 nAb further reduced Arg-1 levels in DOX-treated mice (Figure 4A). In addition, staining for surface markers showed that the expression levels of heart CD80 and CD206 exhibited trends similar to those observed for the expression of iNOS and Arg-1, respectively, in the heart (Figure 4B). Inflammatory cytokine mRNA levels were also investigated, and the results showed that the anti-IL-5 nAb increased the DOX-induced increases in the expression levels of IL-1β, IL-6, IL-17, TNF-α, IFN-γ (Figure 4C) and further reduced the DOX-induced decreases in the mRNA levels of IL-4, IL-10, and IL-13 (Figure 4D).

**Anti-IL-5 nAb increases DOX-induced myocardial apoptosis**

The mRNA levels of apoptotic markers were measured in the LV, and the results showed that the anti-IL-5 nAb
increased the mRNA levels of Bax and Caspase3 but reduced the mRNA levels of Bcl2 in DOX-treated mice (Figures 5A–C). The protein level of caspase 3 was also investigated, and the results showed that DOX treatment significantly increased heart caspase 3 levels, which were further increased by treatment with the anti-IL-5 nAb (Figure 5D). In addition, TUNEL staining showed that apoptotic cells exhibited a trend similar to that observed for the expression of caspase3 in the heart (Figure 5E).

**Anti-IL-5 nAb promotes Mø1 differentiation and MCM cell apoptosis in vitro**

The mRNA levels of iNOS and Arg-1 were measured in Mø, and the results showed that treatment with an anti-IL-5 nAb significantly increased iNOS mRNA levels but decreased Arg-1 mRNA levels in DOX-treated Mø (Figure 6A). In addition, cytokines were detected in the supernatants consisting of the culture medium, and the results showed that the mRNA levels of IL-1β, IL-6, IL-17, TNF-α, and IFN-γ exhibited trends that were similar to those observed for iNOS mRNA levels, while the concentrations of IL-4, IL-10, and IL-13 showed trends similar to those observed for Arg-1 mRNA levels (Figure 6B). Furthermore, the supernatants obtained from the culture medium above were used to determine the effect of the anti-IL-5 nAb on DOX-induced apoptotic marker secretion in MCM cells, and the results showed that the Bax and Cle-cas3 levels were increased while the protein levels of Bcl2 were decreased by the anti-IL-5 nAb in DOX-treated MCM cells (Figure 6C).

**Discussion**

In the present study, we first investigated the effects of DOX on IL-5 expression in the heart and found that treatment with DOX for 5 days significantly reduced the IL-5 levels by decreasing cardiac-Mø-derived IL-5 expression. We then determined whether the anti-IL-5 nAb regulated DOX-induced cardiac injury, and the results showed that anti-IL-5 nAb treatment increased cardiac injury marker levels and aggravated cardiac dysfunction. Treatment with anti-IL-5 nAb also exacerbated the imbalance between Mø1 and Mø2, increased heart apoptotic mRNA expression levels, and promoted myocardial cell apoptosis. Furthermore, anti-IL-5 nAb was used to determine its effects on Mø differentiation and myocardial cell apoptosis in vitro, and we found that anti-IL-5 nAb treatment promoted Mø1 but inhibited Mø2 differentiation and alleviated DOX-induced MCM cell apoptosis.
Our results show that anti-IL-5 nAb-aggravated DOX-induced cardiac injury by further enhancing the inflammatory response and increasing myocardial cell apoptosis.

Numerous studies have demonstrated that IL-5 is closely related to the occurrence of cardiovascular diseases. In an earlier study, Cappuzzello et al. (2011) reported that serum IL-5 levels were significantly lower in chronic heart failure patients than in control subjects. In addition, IL-5 was also reported to be decreased in an angiotensin II-induced atherosclerosis model, and exogenous IL-5 treatment alleviated atherosclerosis development in both atherosclerosis mouse model, and exogenous IL-5 treatment alleviated atherosclerosis development in both apoipoprotein E knockout and LDL receptor-multipotent alleviated atherosclerosis development in both apoipoprotein E knockout and LDL receptor-multipotent mitigated atherosclerosis development in both apoipoprotein E knockout and LDL receptor-multipotent an apoipoprotein E knockout and LDL receptor-multipotent mice (Meng et al., 2015; Zhao et al., 2015). In the present study, we measured cardiac IL-5 mRNA expression levels in inducible nitric oxide synthase (iNOS) and arginase-1 (Arg-1) in the four groups. (B) M1 macrophage (Mø1) and M2 macrophage (Mø2)-related cytokine levels were measured in the supernatant of each group. (C) The protein levels of Bax, Bcl2, and Cle–cas3 were measured in MCM cells. N = 5 in each group. *P < 0.5 versus the Saline + nAb (anti-IL-5 nAb) group and #P < 0.5 versus the doxorubicin (DOX) + nAb group. GADPH, glyceraldehyde 3-phosphate dehydrogenase; IgG, immunoglobulin G.

To investigate the role of IL-5 in cardiac injury, DOX-treated mice were administered the anti-IL-5 nAb in advance. We found that pre-treatment with anti-IL-5 nAb significantly reduced body weight and heart weight while increasing vacuolated cardiomyocyte numbers and the expression of both heart and serum heart myocardial injury markers. In addition, DOX-induced cardiac dysfunction was worsened by anti-IL-5 nAb. These findings demonstrate that the anti-IL-5 nAb further exacerbated DOX-induced cardiac injury.

Mø is the most important inflammatory cells, and they participate in a variety of diseases by regulating the inflammatory response. Mature Mø can be divided into Mø1, which play a pro-inflammatory role and secrete a variety of pro-inflammatory cytokines such as IL-1β, IL-6, IL-17, TNF-α, and IFN-γ and Mø2, which play anti-inflammatory roles and secrete a variety of anti-inflammatory cytokines such as IL-4, IL-10, and IL-13. Imbalances between Mø1 and Mø2 levels have been used to explore multiple cardiovascular diseases such as abdominal aortic aneurysm and aortic dissection (Son et al., 2015;
Yahagi et al., 2017; Vozenilek et al., 2018). A recent study also reported that such an imbalance was one of the most important mechanisms underlying DOX-induced cardiac injury because the imbalance between Mø1 and Mø2 resulted in a strong inflammatory response, which led to apoptosis in myocardial cells (Ye et al., 2018). In addition, IL-5 has also been previously reported to affect Mø-related mRNA expression levels (Meng et al., 2015). To explore the mechanism by which anti-IL-5 nAb-aggerrated DOX-induced cardiac injury, both surface markers and intracellular markers of Mø1 and Mø2 were detected, and the results showed that treatment with an anti-IL-5 nAb increased the expression levels of surface and intracellular markers of Mø1 and decreased the mRNA levels of surface and intracellular markers of Mø2. These results showed that the anti-IL-5 nAb promoted Mø1 differentiation but inhibited Mø2 differentiation in DOX-treated mice. This increase in the imbalance between Mø1 and Mø2 is one mechanism by which anti-IL-5 can aggravate DOX-induced cardiac injury.

The inflammatory response is not the only mechanism of DOX-induced cardiac injury. Both higher oxidative stress and higher antioxidant activity were observed in DOX-treated mice, and the literature overwhelmingly indicates that oxidative stress makes the heart uniquely vulnerable to oxidative stress (Xu et al., 2001; Ye et al., 2018). Autophagy induced by endoplasmic reticulum stress always has a dual effect on cardiac injury because mild autophagy provides some protection to myocardial cells against harmful conditions and promotes cell survival, whereas severe or rapid autophagy induces programmed cell death via a mechanism known as autophagic cell death (Gump and Thorburn, 2011; Jing and Lim, 2012; Ryter et al., 2014; Ye et al., 2018), and rapid autophagy has also been observed in the hearts of DOX-treated mice (Ye et al., 2018). Although many different mechanisms participate in DOX-induced cardiac injury, the evidence described above shows that the essential mechanism underlying DOX-induced myocardial injury is excessive apoptosis of myocardial cells, and has been demonstrated by a variety of studies (Ye et al., 2018; Yuan et al., 2018). To further explore the mechanisms of anti-IL-5 nAb-aggravated DOX-induced cardiac injury, we first detected the mRNA levels of apoptotic markers, and the results showed that treatment with anti-IL-5 nAb induced higher Bax mRNA levels and lower Bcl2 levels. Cle-cas3 plays an essential role in apoptosis; therefore, we also measured mRNA and protein levels of Cle-cas3 in the heart. We found that treatment with anti-IL-5 nAb directly induced more positive apoptotic cells in the heart. This evidence demonstrates that myocardial apoptosis is another mechanism of anti-IL-5 nAb-aggravated DOX-induced cardiac injury. In addition, anti-IL-5 nAb promoted Mø1 differentiation and increased Mø1-related cytokine levels in the culture supernatants while also inhibiting Mø2 differentiation and decreasing Mø2-related cytokines. The supernatants collected from anti-IL-5 nAb-treated Mø also aggravated the mRNA levels of apoptotic markers in MCM cells. These results suggest that the anti-IL-5 nAb-aggravated DOX-induced cardiac injury and myocardial cell apoptosis by regulating Mø differentiation and amplifying inflammation.

In summary, the results of the present study are the first to demonstrate that an anti-IL-5 nAb-aggravated DOX-induced cardiac injury and cardiac dysfunction. Although the exact mechanism by which IL-5 acts in DOX-induced cardiac injury has not been fully clarified, the upregulation of the inflammatory response and the increase observed in myocardial cell apoptosis are potential underlying mechanisms. Consequently, our results show that IL-5 is a potential new therapeutic option for the clinical prevention and treatment of DOX-induced cardiac injury.

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