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RESEARCH ARTICLE

Oral administration of ammonium metavanadate and potassium dichromate distorts the inflammatory reaction induced by turpentine oil injection in male rats

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ABSTRACT

Heavy metal pollution is rapidly increasing in the environment. It has been shown that exposure to vanadium and chromium is able to alter the immune response. Nevertheless, the mechanisms by which these metal pollutants mediate their immunomodulatory effects are not completely understood. Herein, we examined the effect of ammonium metavanadate and potassium dichromate on the development of an inflammatory response caused by subcutaneous injection of turpentine oil. We demonstrated that pretreatment of rats with ammonium metavanadate and potassium dichromate for two weeks prior to initiation of the inflammatory response resulted in a wider zone of necrosis surrounding the site of inflammation. The acute inflammatory process in the combined model was characterized by elevated serum levels of IL-10 and decreased serum levels of IL-6 as compared to rats not treated with ammonium metavanadate and potassium dichromate. Ammonium metavanadate and potassium dichromate administration induced a decrease in the proportion of splenic $His48^{High}CD11b/c^{+}$ myeloid cells accompanied by a reduced infiltration of the wound with neutrophils. Further analysis showed decreased proportions of $CD3^{+}CD4^{+}IFN\gamma^{+}$ and $CD3^{+}CD4^{+}IL\text{-}4^{+}$ T cells in the rats with combined model as compared to inflamed rats not treated with ammonium metavanadate and potassium dichromate. The data suggest that consumption of vanadium and chromium compounds disrupts the inflammatory response through an altered balance of pro- and anti-inflammatory cytokines and inhibition of effector T cell activation and neutrophil expansion.

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Ammonium metavanadate; potassium dichromate; aseptic inflammation; myeloid cells; T cells

1. Introduction

Heavy metals are present in the air, drinking water, food, and countless synthetic chemicals and products. Heavy metals taken into the body by inhalation, ingestion, and skin absorption are able to accumulate in body tissues. Being highly toxic, heavy metals demonstrate direct, primary or secondary effects on the immune system resulting in increased susceptibility to infections, a wide variety of hypersensitivity reactions, autoimmune diseases, and neoplasia (Shrivastava *et al.* 2002). Chromium and vanadium are two widely spread metal-pollutants. An excess of these compounds, when interacting with biological molecules, disrupts cell functions and initiates tissue damage and cell death (Miller *et al.* 2004).

It has been shown that prolonged exposure to chromium and vanadium was associated with chronic inflammatory conditions and an increased risk for several types of cancer (Aragón *et al.* 2005, Avila-Costa and Fortoul 2007, Chandra *et al.* 2007, Fortoul *et al.* 2007, Piñon-Zarate *et al.* 2007, Shelnutt *et al.* 2007, Chen *et al.* 2009, Sa *et al.* 2016). Chromium is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (Boffetta 1993). Vanadium-containing compounds were also found to exert potent toxic and carcinogenic effects (Morinville *et al.* 1998). The association between exposure to chromium and vanadium and chronic inflammation and carcinogenesis has been extensively studied. Nevertheless, the mechanisms of their immunotropic action are far from being completely understood. A series of *in vitro* and *in vivo* studies demonstrated that chromium and vanadium were able to inhibit numbers and functional activity of neutrophils (Cohen *et al.* 2010), decrease production of pro-inflammatory cytokines and expression of receptors to them by immune cells, as well as inhibit immune cell infiltration of the lesion site (Cui *et al.* 2011, Tlili *et al.* 2015). Exposure to chromium and vanadium resulted in decreased levels of serum IgG, IgA, IgM, transferrin (acute-phase protein), α1-antitrypsin, β2-microglobulin, haptoglobin, ceruloplasmin, and

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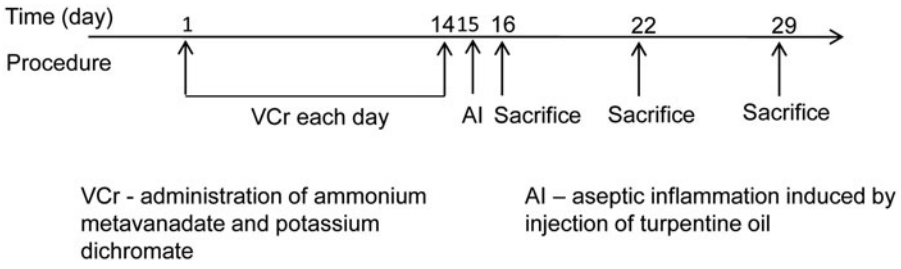

Figure 1. Flow chart of experimental interventions.

down-regulated cytotoxicity of lymphokine-activated NK cells (Zeidler-Erdely *et al*. 2012).

Thus, while it is clear that chromium and vanadium are able to alter a number of immune parameters, the effects of these metals on the immune response during acute inflammation have not been investigated. Moreover, it is unclear what mechanisms triggered by these heavy metals result in the arrest of the resolution of inflammation and lead to it becoming a chronic condition. To better understand the involved regulatory mechanisms, we investigated the development of aseptic inflammation in rats that were preliminarily treated with ammonium metavanadate and potassium dichromate.

2. Materials and methods

2.1. Rats

The study was conducted on male rats with body weight of 180–220 g, contained in standard vivarium conditions and fed on a standard diet. For all performed experiments animals were handled in strict accordance with good animal practice as defined by the relevant international and state animal welfare regulations. Rats were maintained under conventional conditions (room temperature 22.5–23.0 °C, relative humidity 50–70%, 12 h day/night cycle) with free access to food and water. All experiments were approved by the ethical committee of S.D. Asfendiyarov Kazakh National Medical University (protocol # 3 of 01/04/2015).

The animals were randomly divided into 4 groups of 6 animals each: control animals (CA group), animals with induced aseptic inflammation (AI), animals that received metal compounds - ammonium metavanadate (NH_4VO_3) and potassium dichromate ($K_2Cr_2O_7$) (VCr group), and animals that were first exposed to ammonium metavanadate and potassium dichromate for two weeks, followed by modeling aseptic inflammation (VCr/AI group). Ammonium metavanadate and potassium dichromate were dissolved in phosphate-buffer saline (PBS) and administered orally *via* an esophageal catheter at a dose of 5 mg/kg of body weight each day for two weeks as described previously (Rasool *et al*. 2014, Eiam-Ong *et al*. 2018, Koubaa *et al*. 2018). The CA and AI groups received an equal volume of PBS orally. To model aseptic inflammation, rats were anesthetized with chloroform inhalation, after that the interscapular region was shaved, treated with 70% ethanol, and 0.3 ml of turpentine oil was injected subcutaneously. On day 1, 7, and 14 after injection of turpentine oil blood samples were taken by terminal retro-orbital bleeding under chloroform anesthesia and the rats were euthanized (Figure 1). Thymus and spleen were removed from sacrificed rats. The thymus was homogenized in PBS by a tissue grinder, and cellularity of the thymus was estimated as a cell quantity per mg of the thymus tissue weight.

2.2. Histopathological evaluation

The tissue specimens obtained from the site of inflammation were fixed with 10% neutral formalin or Carnoy's fixative (cat. no. R1851000, Ricca Chemical Company) and processed according to the standard histological methods. 5–7-micron paraffin-embedded sections were stained with hematoxylin-eosin (cat. no. 1043020025, Merck) and picrofuchsin (cat. no. 100199, Millipore) according to the van Gieson technique. The preparations were studied with a light microscope Axio Lab A1 with a digital camera QImaging QICam (Carl Zeiss Microscopy GmbH) at 200× magnification.

In all experimental groups, a blinded semi-quantitative 4-point scale from 0 to 3 (0 = none, 1 = weak, 2 = moderate, 3 = strong) was assigned to calculate the severity of the ongoing aseptic inflammation according to the following pathological findings: the size of a necrotic zone, swelling of soft tissues, leukocyte infiltration, proliferation of blood vessels and fibroblasts (Ahmadi *et al*. 2018, Keyhanmanesh *et al*. 2018).

2.3. Preparation of the splenocyte suspension

Splenocytes were obtained by homogenization of the spleen in PBS by a tissue grinder. Contaminating erythrocytes were lysed with lysing solution (0.83% NH_4Cl, 0.1% $KHCO_3$, 0.003% EDTA, pH = 7.2–7.4) for 10 min at room temperature. Cells were then washed, filtered through 30 μm pre-separation filters (cat. no. 130–041-407, Miltenyi Biotec) and re-suspended in PBS.

2.4. In vitro *activation of CD4$^+$ T cells*

Splenocytes were resuspended at the concentration of 2×10^6 cells/ml in RPMI-1640 (cat. no. R6504-10X1L, Sigma-Aldrich) supplemented with 10% FCS, 100 U/ml penicillin, 100 mg/ml streptomycin (cat. no. P0781, Sigma), and 2 mM L-glutamine (cat. no. G2150, Sigma). Phorbol 12-myristate 13-acetate (PMA, 10 ng/ml) (cat. no. P1585, Sigma) and ionomycin (500 ng/ml) (cat. no. 56092–81-0, Sigma-Aldrich)

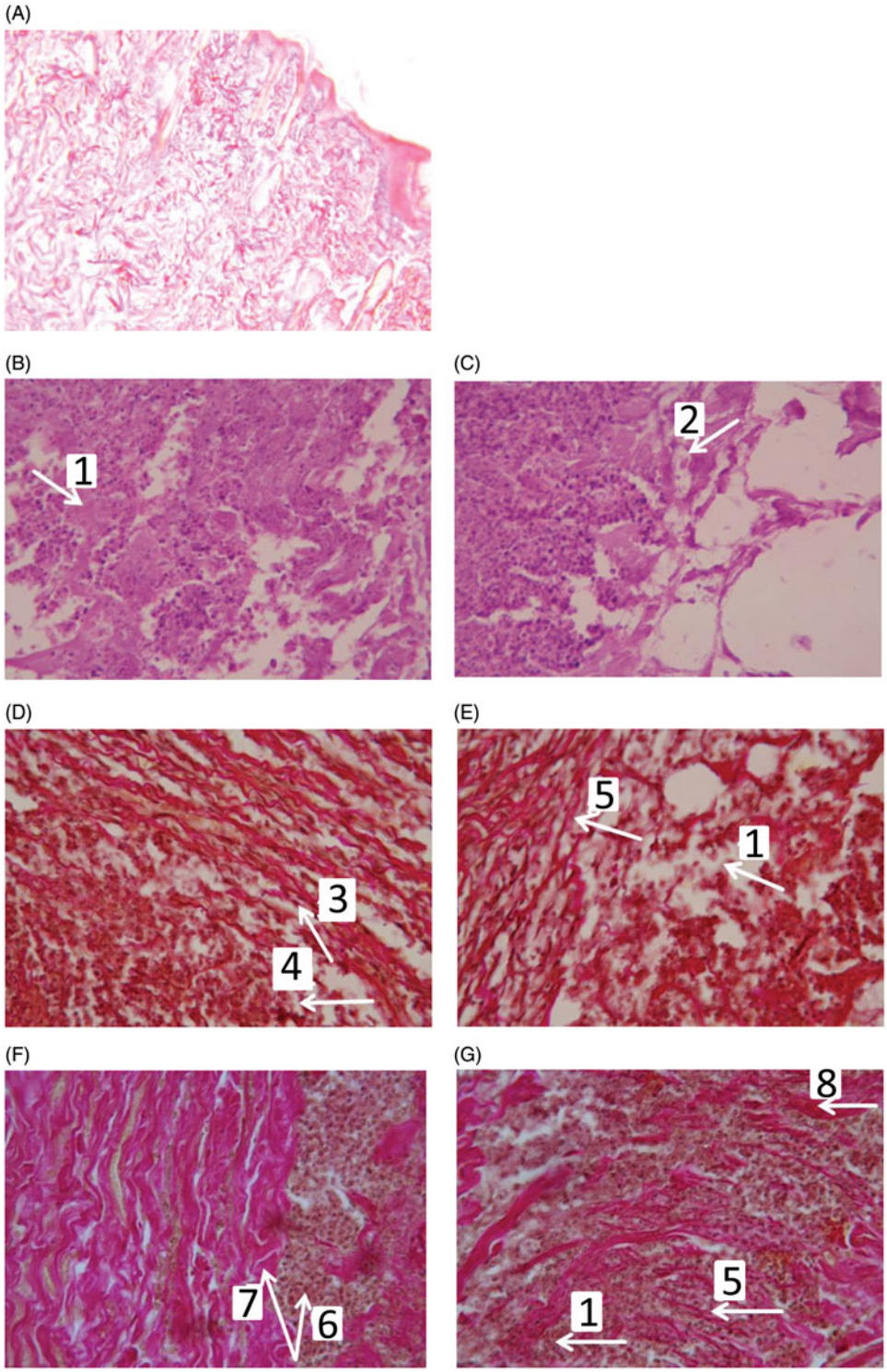

Figure 2. Exposure to ammonium metavanadate and potassium dichromate prolongs tissue regeneration in rats with aseptic inflammation. Aseptic inflammation was induced by a subcutaneous injection of turpentine oil into the intact rats (AI group) and rats that were pretreated with ammonium metavanadate and potassium dichromate for 2 weeks (VCr/AI group). The rats were sacrificed in 1, 7 and 14 days after initiation of aseptic inflammation. Inflamed tissues were fixed in paraffin and stained with hematoxylin-eosin and picro fuchsin by van Gieson. Representative images of 6 independent experiments are shown. Histological slides for the CA group (A), AI group on day 1 (B), day 7 (D), and day 14 (F) and VCr/AI group on day 1 (C), 7 (E) and day 14 (G) are shown. Arrows indicate: 1 – necrosis; 2 – edema; 3 – granulation tissue zone; 4 – exudate resorption; 5 – edematous granulation tissue; 6 – remains of necrotic tissue; 7 – fibrous capsule; 8 – fibrosis zone. Original magnification, ×200.

were added as described previously (Debes *et al*. 2002) to stimulate cytokine expression. Cells were incubated in 96-well plates for 4 h in a humidified CO_2 incubator at 37 °C. Brefeldin A (cat. no. 420601, Biolegend) was added according to the manufacturer's protocol (1 μl/ml) for the last hour of cultivation to prevent cytokine efflux and the cells were cultured for an additional hour. The intracellular expression of IFN-γ and IL-4 and the surface expression of T helper (Th) markers (CD3, CD4) were assessed by flow cytometry.

Table 1. Pathological scores in the skin tissues of CA, AI and VCr/AI animals after 1, 7 and 14 days after initiation of aseptic inflammation.

	Pathological findings			
	Necrotic zone	Soft tissue swelling	Leukocyte infiltration	Proliferation of blood vessels and fibroblasts
Scores in groups (for each group, $n = 6$)	(Minimum–maximum)			
	Day 1			
CA	(0–0)	(0–0)	(0–0)	(0–0)
AI	(2–3)*	(2–3)*	(2–3)*	(0–0)
VCr/AI	(3–3)*	(3–3)*	(0–1)* +	(0–0)
	Day 7			
CA	(0–0)	(0–0)	(0–0)	(0–0)
AI	(2–3)*	(1–2)*	(2–3)*	(1–2)*
VCr/AI	(2–3)*	(2–3)* +	(1–2)* +	(0–1)*
	Day 14			
CA	(0–0)	(0–0)	(0–0)	(0–0)
AI	(0–1)*	(0–1)*	(3–3)*	(2–3)*
VCr/AI	(1–2)* +	(1–2)* +	(2–3)*	(1–2)* +

NOTE: significant differences assessed by one-way ANOVA analysis are defined as: *$p < 0.01$ in comparison with the CA group; +$p < 0.01$ in comparison with the respective AI group. The minimal–maximal pathological values in each group are placed between the parentheses.

2.5. Flow cytometry

The following mouse monoclonal antibodies (mAbs) were used for surface staining: APC-labeled anti-CD3 (cat. no. 557030, BD Biosciences), PE-Cy5-labeled anti-CD4 (cat. no. 554839, BD Biosciences), PerCP-labeled anti-CD8a (cat. no. 558824, BD Biosciences), FITC-labeled anti-His48 (cat. no. 554907, BD Biosciences), PE-labeled anti-CD11b/c (cat. no. 554862, BD Biosciences), PE-labeled anti-IL-4 (cat. no. 555082, BD Biosciences), and FITC-labeled anti-IFNγ (cat. no. 559498, BD Biosciences) were used for intracellular staining. Briefly, 10^6 cells were incubated with mAbs specific for surface markers according to the manufacturer's protocols, then fixed and permeabilized with Fixation/Permeabilization solution (cat. no. 554722, BD Biosciences) for 20 min in the dark at room temperature. Cells were then washed with Perm/Wash Buffer (cat. no. 554723, BD Biosciences) and stained with mAbs specific for intracellular cytokines. Afterward, cells were washed with PBS, re-suspended in flow solution, and immediately analyzed by flow cytometry on a FACSCalibur (BD Biosciences) using CellQuest Pro software (BD Biosciences).

Unstained cells, single fluorochrome stained cells, and cells stained as fluorescence-minus-one controls were used to set-up the flow cytometer. Multiparameter data were analyzed as described previously (Carleton and Nicholson 2000).

2.6. ELISA

Serum levels of IL-1β, IL-6, TGF-β, and IL-10 in control and experimental rats were analyzed by ELISA using the commercially available kits: IL-1β (cat. no. RLB00), IL-6 (cat. no. R6000B), TGF-β (cat. no. RLB00), and IL-10 (cat. no. R1000) Rat ELISA Kits (R&D Systems). The mean absorbance was calculated from the standard curve.

2.7. Statistical analysis

Variables were analyzed by a one-way ANOVA analysis with Tukey–Kramer *post hoc* test, and a $p < 0.05$ was considered statistically significant. Values are expressed as mean ± SD of at least six independent experiments. GraphPad Prism 4 was used to create and design data graphics.

3. Results

3.1. Exposure to ammonium metavanadate and potassium dichromate prolongs tissue regeneration in aseptic inflammation

Histological analysis was undertaken to determine the effects of ammonium metavanadate and potassium dichromate on the course of aseptic inflammation (Figure 2, Table 1).

Pathological changes in the inflamed tissues of AI and VCr/AI groups at all-time points were significantly higher as compared to the control group ($p < 0.01$). On day 1 after initiation of aseptic inflammation, VCr/AI group was characterized by a weaker leukocyte infiltration in the site of inflammation as compared to the AI rats ($p < 0.01$). On day 7, soft tissue swelling was significantly more severe in the VCr/AI group as compared to AI rats ($p < 0.01$). In 14 days after initiation of inflammation, VCr/AI group was characterized by persistence of necrosis foci and tissue swelling, while the score of the proliferation of small vessels and fibroblasts was significantly lower than in the AI group ($p < 0.01$) (Figure 2, Table 1).

Analysis of the thymus demonstrated that a 2-week administration of ammonium metavanadate and potassium dichromate resulted in a nearly 2-fold decrease in the cellularity as compared to untreated rats ($1.0 \pm 0.5 \times 10^6$ cells/mg in VCr group and $2.0 \pm 0.5 \times 10^6$ cells mg in CA group, $p = 0.02$).

Thus, turpentine oil induced a classic inflammatory reaction with resolution in 14 days characterized by resorption of exudate and formation of a connective tissue capsule, whereas pretreatment of rats with ammonium metavanadate and potassium dichromate significantly slowed down proliferative processes in the inflamed tissue, delayed the resolution of inflammation, and led to destructive changes in the thymus.

3.2. Exposure to vanadium and chromium results in an altered balance of pro-inflammatory and anti-inflammatory cytokines in rats with aseptic inflammation

To study the effect of ammonium metavanadate and potassium dichromate on the balance between pro- and

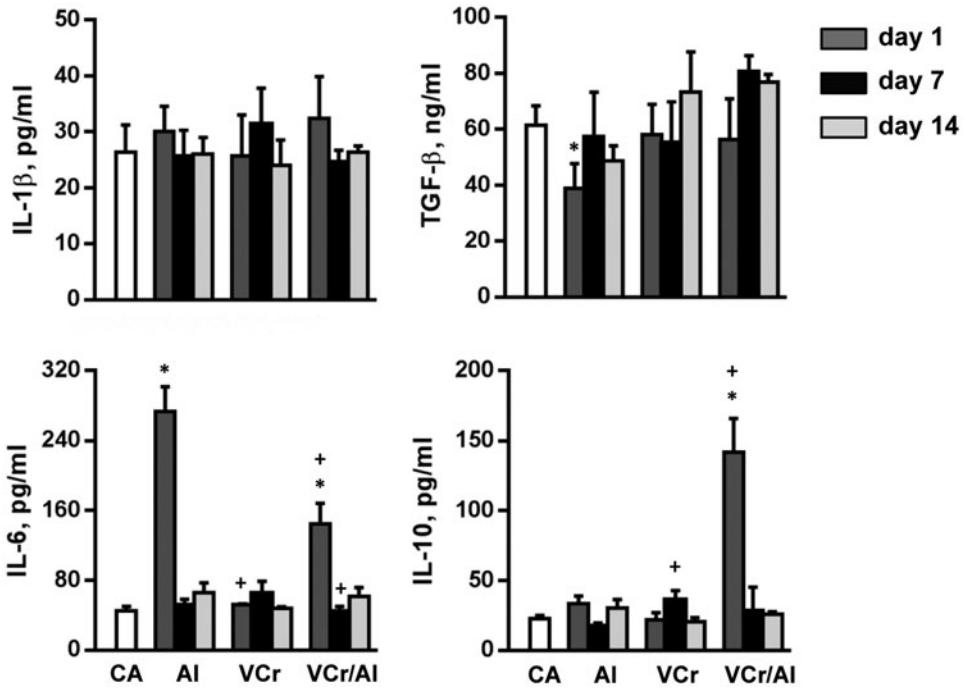

Figure 3. Exposure to ammonium metavanadate and potassium dichromate results in decreased sera levels of pro-inflammatory IL-6 and increased sera levels of anti-inflammatory IL-10 in the acute phase of aseptic inflammation. Levels of IL-1β, IL-6, TGF-β1, and IL-10 were examined by ELISA in the serum of CA, AI, VCr, and VCr/AI rats. *p* values calculated using the one-way ANOVA analysis with Tukey–Kramer *post hoc* test are indicated as: $^{*}p < 0.05$ in comparison with CA; $^{+}p < 0.05$ in comparison with the respective AI group.

anti-inflammatory cytokines during the development of inflammation, we determined the levels of IL-1α, IL-6, TGF-β, and IL-10 cytokines in AI and VCr/AI rats in 1, 7, and 14 days after initiation of aseptic inflammation. CA and VCr rats in 1, 7, and 14 days after the last exposure to ammonium metavanadate and potassium dichromate were used as controls.

On day 1 after induction of aseptic inflammation, we detected an increased level of serum IL-6 in AI rats as compared to CA, VCr/AI and VCr groups. In rats pretreated with ammonium metavanadate and potassium dichromate, the inflammatory reaction in the acute phase was characterized by weaker IL-6 production when compared to inflamed rats not subjected to ammonium metavanadate and potassium dichromate (Figure 3). Moreover, on day 1 after turpentine oil injection, we observed an increase in the level of serum IL-10 in VCr/AI rats as compared to CA, AI and VCr rats. Serum levels of IL-1β and TGF-β did not differ significantly among the groups during the experiment (Figure 3).

Together these data demonstrate that exposure to ammonium metavanadate and potassium dichromate shifts the production of pro-inflammatory IL-6 to the production of anti-inflammatory IL-10 during the acute phase of inflammation.

3.3. Exposure to vanadium and chromium interferes with expansion of neutrophils in rats with sterile inflammation

Neutrophils and monocytes/macrophages are key players in inflammatory reactions (Prame Kumar *et al*. 2018). Therefore, we tested the effect of ammonium metavanadate and potassium dichromate on the expansion of neutrophils defined as $His48^{High}CD11b/c^{+}$ and monocytes defined as $His48^{low}CD11b/c^{+}$ in AI (Barnett-Vanes *et al*. 2016).

Sterile inflammation significantly up-regulated levels of splenic $His48^{High}CD11b/c^{+}$ cells in AI rats in 7 and 14 days after injection of turpentine oil. Pretreatment with ammonium metavanadate and potassium dichromate abolished systemic accumulation of $His48^{High}CD11b/c^{+}$ cells in VCr/AI rats as observed on day 7 after turpentine oil injection (Figure 4). We did not detect significant differences in the proportion of $His48^{low}CD11b/c^{+}$ splenocytes between the AI and VCr/AI groups (Figure 4).

Together, these data demonstrate that administration of ammonium metavanadate and potassium dichromate interferes with expansion of neutrophils in animals with aseptic inflammation.

3.4. Pretreatment with ammonium metavanadate and potassium dichromate inhibits cytokine production by effector T cells in rats with aseptic inflammation

Given the fact that exposure to ammonium metavanadate and potassium dichromate-induced destructive changes in the thymus, we next examined T cell activation. For that, we analyzed the frequency of T cells in the spleen and their expression of IFNγ and IL-4 after *in vitro* stimulation.

The levels of $CD8^{+}$ cells did not differ significantly between the groups at all time points (data not shown). Injection of turpentine oil triggered an increase in the proportion of $CD4^{+}$ cells in the acute phase (day 1 after induction of inflammation), followed by a decline to the level of control groups on day 7 and 14. The same trend was

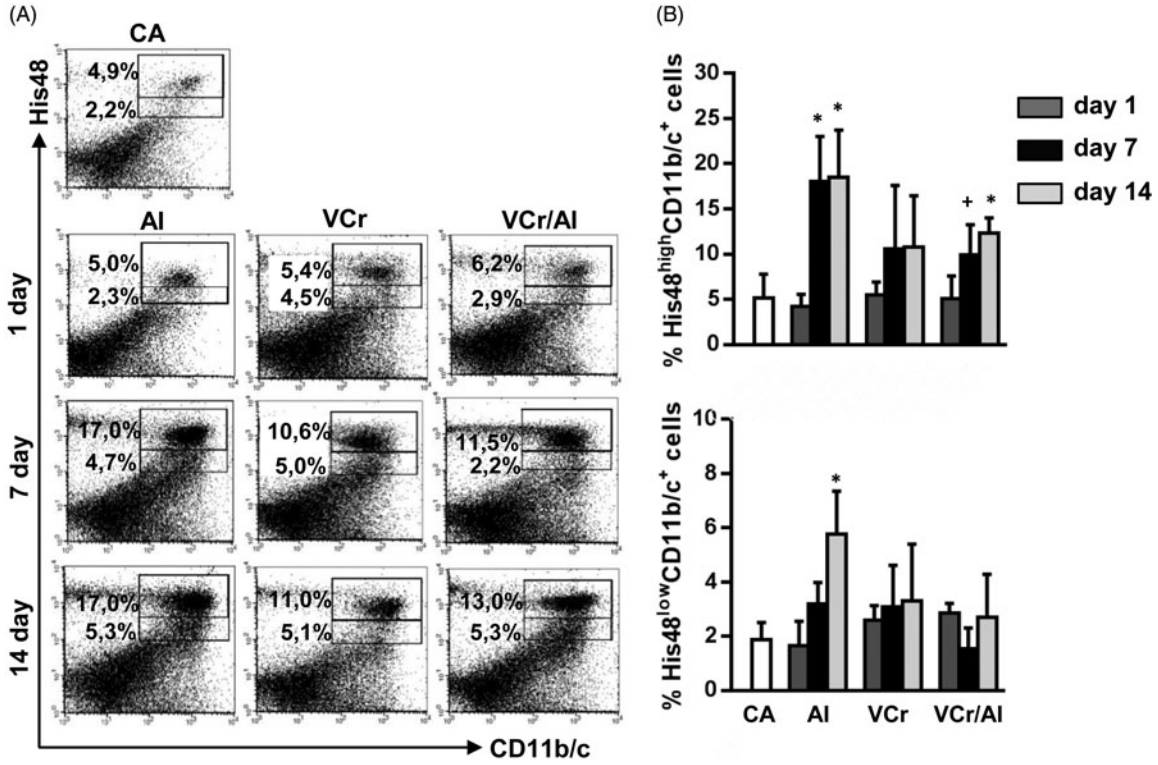

Figure 4. Pretreatment with ammonium metavanadate and potassium dichromate results in the decreased proportion of His48^{High}CD11b/c^{+} myeloid cells in the spleen of rats with aseptic inflammation. Splenocytes from CA, AI, VCr and VCr/AI rats were labeled with anti-His48 and anti-CD11b/c and the frequency of His48^{High}CD11b/c^{+} and His48^{low}CD11b/c^{+} cells was analyzed on day 1, 7 and 14 after initiation of aseptic inflammation. Each group included at least 6 rats. Representative flow cytometry results (A) and cumulative results for each group (B) are shown. Significant differences between columns assessed by one-way ANOVA analysis with Tukey–Kramer *post hoc* test are indicated as: $*p < 0.05$ in comparison with the CA group and $+p < 0.05$ in comparison with the respective AI group.

observed in the VCr/AI group. Ammonium metavanadate and potassium dichromate alone did not affect the frequency of CD4^{+} cells (Figure 5).

Further cytofluorimetric analysis demonstrated that aseptic inflammation triggered increased production of both IFNγ and IL-4 by CD4^{+} cells in AI rats in 7 and 14 days after injection of turpentine oil (Figure 5). Exposure to ammonium metavanadate and potassium dichromate significantly suppressed the production of IFNγ and IL-4 cytokines by CD4^{+} cells triggered by turpentine oil injection (Figure 5).

Collectively, these data indicate that ammonium metavanadate and potassium dichromate are able to alter the course of inflammation *via* inhibition of IFNγ and IL-4 production.

4. Discussion

In the classical variant, the acute phase of inflammation is characterized by release of pro-inflammatory cytokines (TNFα, IL-1, IL-6, IL-8, and IL-12) produced by macrophages. The cytokines induce increased vascular permeability and rapid migration of neutrophils and monocytes into the inflamed tissue (Bagaitkar 2014). When macrophages engulf a foreign antigen, they process it and represent on the cell membrane, where they will be recognized by Th cells, which release cytokines that activate B cells. Activated B lymphocytes then secrete specific antibodies, which attach to the antigens and promote their more efficient phagocytosis (Arango Duque and Macrophage 2014). After antigen clearance, anti-inflammatory cytokines such as IL-4, IL-10 and TGFβ, resolution-promoting macrophages, proteins of the lipoxygenase family, as well as lipid mediators like lipoxins and E- and D-series resolvins promote the resolution of inflammation (Ortega-Gómez *et al*. 2013, Rombouts *et al*. 2016). Thus, an acute inflammatory response is beneficial for restoration and maintenance of the host tissue homeostasis. On the other hand, chronic inflammation can lead to chronic organ failure, a decrease in the quality of life and, ultimately, a shortened life span.

It has been shown that prolonged exposure to chromium and vanadium is associated with chronic inflammatory conditions (Aragón *et al*. 2005, Avila-Costa and Fortoul 2007, Chandra *et al*. 2007, Fortoul *et al*. 2007, Piñón-Zarate *et al*. 2007, Shelnutt *et al*. 2007, Chen *et al*. 2009, Sa *et al*. 2016), nevertheless, the mechanisms behind the transition to chronic inflammation mediated by these metals are not well understood. The present study investigated the effect of ammonium metavanadate and potassium dichromate on the development of local acute inflammation, induced by injection of turpentine oil. We showed that the inflammatory process in AI rats was characterized by up-regulated production of IL-6 in the initial phase and a large number of infiltrating neutrophils in the inflamed tissue followed by normal healing in 14 days after turpentine oil injection. Pretreatment of rats with ammonium metavanadate and potassium dichromate before initiation of inflammation resulted in insufficient wound infiltration by neutrophils in the initial inflammatory phase associated with a wider zone of necrosis, low exudate

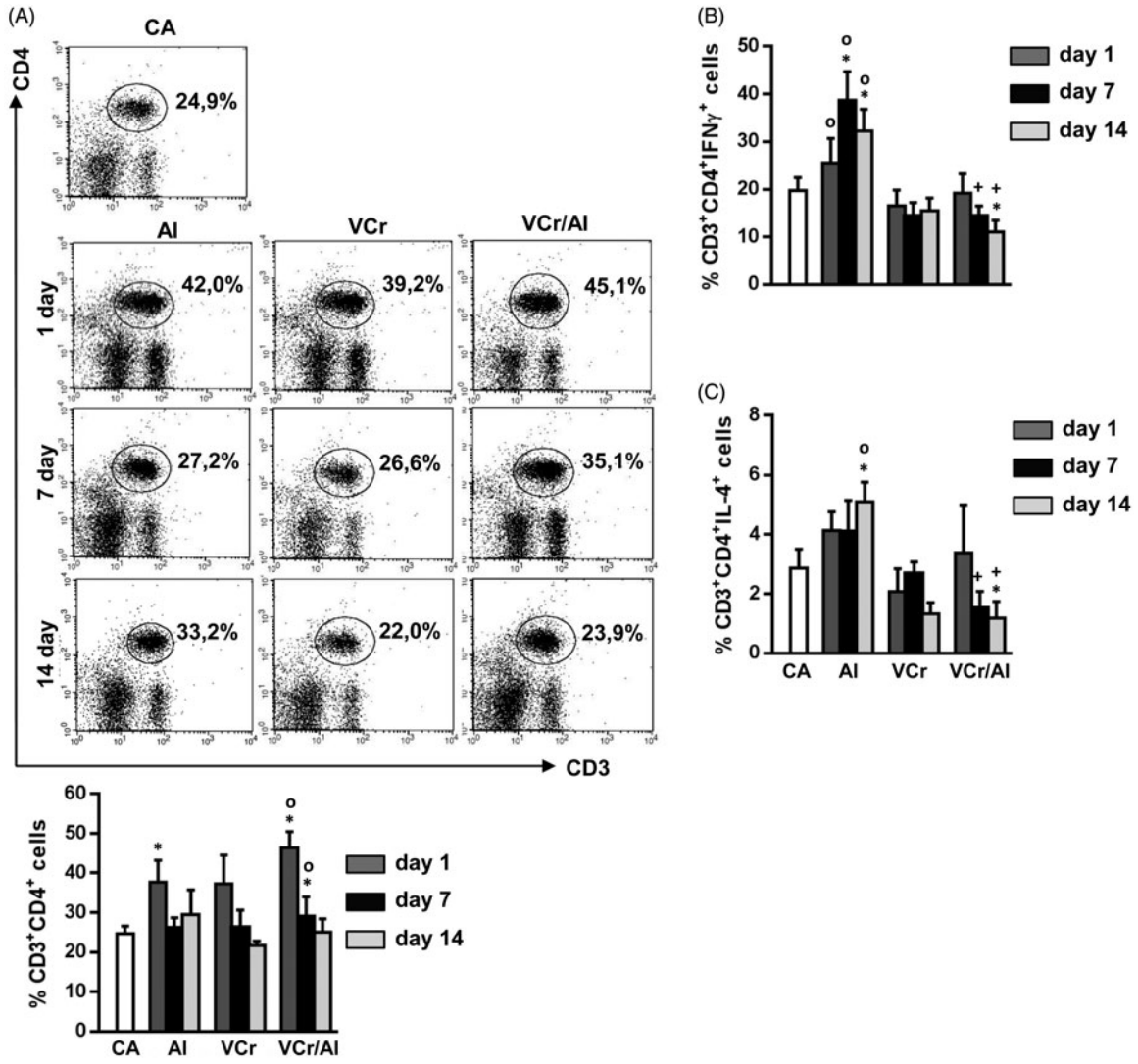

Figure 5. Pretreatment with ammonium metavanadate and potassium dichromate results in decreased proportions of $CD3^+CD4^+IFN\gamma^+$, and $CD3^+CD4^+IL\text{-}4^+$ cells in the spleen of rats with aseptic inflammation. Splenocytes from CA, AI, VCr, and VCr/AI rats were labeled with anti-CD3 and anti-CD4 and the frequency of (A) $CD3^+CD4^+$cells was analyzed in the lymphocyte gate on day 1, 7 and 14 after initiation of aseptic inflammation. Also, splenocytes were stimulated with PMA and ionomycin for 4 h, stained with anti-CD3, anti-CD4, anti-IFNγ, and anti-IL-4 and the frequency of (B) $CD3^+CD4^+IFN\gamma^+$ and (C) $CD3^+CD4^+IL\text{-}4^+$ cells was analyzed in the lymphocyte gate on day 1, 7 and 14 after initiation of inflammation. Each group included 6 rats. Significant differences between columns assessed by one-way ANOVA analysis with Tukey–Kramer *post hoc* test are indicated as: $^*p < 0.05$ in comparison with the CA group; $+p < 0.05$ in comparison with the respective AI group; $^\circ p < 0.05$ in comparison with the respective VCr group.

dissolution and delayed healing. The obtained results are consistent with an earlier report showing that insufficient neutrophil infiltration delays wound repair (Khaled *et al.* 2013). The poor wound infiltration by neutrophils was also accompanied by reduced numbers of $His48^{High}CD11b/c^+$ neutrophils and $His48^{low}CD11b/c^+$ monocytes in the spleen of VCr/AI rats.

We assume that the poor infiltration of the wound tissue by neutrophils in VCr/AI rats was a result of insufficient production of IL-6 in the acute phase of inflammation observed on day 1 and 7 after initiation of inflammation. It is well known that IL-6, which signals *via* the common receptor subunit gp130, is essential for the induction of pro-inflammatory immune responses and represents a crucial checkpoint regulator of neutrophil trafficking during early inflammatory responses (Fielding *et al.* 2008). The data obtained by us are supported by earlier obtained results showing that chromium and vanadium-containing compounds are able to decrease IL-6 production. It was shown that the release of IL-2 and IL-6 by PHA-stimulated human peripheral blood mononuclear cells was inhibited by chromium ($Cr(NO_3)_3$) (Wang *et al.* 1996). Snyder C.A. *et al.* found a significant decrease in the levels of IL-6 in individuals exposed to chromium (Snyder *et al.* 1996). Ammonium metavanadate decreased serum IL-2 and IL-6 content in broilers (Cui *et al.* 2011).

On the other hand, the early inflammatory response in VCr/AI rats was characterized by increased production of IL-10. This cytokine coordinates the phase of resolution due to its ability to tone down the effects of pro-inflammatory cytokines (Khaled *et al.* 2013), and its excessive production during the acute phase of inflammation may lead to insufficient immune responses to an antigen and, thereafter, the development of chronic inflammation (Iyer and Cheng 2012). Similar results were obtained by Snyder C.A. *et al.* (Snyder *et al.* 1996). They demonstrated that treatment with bis (peroxido) vanadium significantly increased levels of IL-10 in the

ischemic boundary zone of the cerebral cortex. The obtained results suggest that ammonium metavanadate and potassium dichromate limit the pro-inflammatory signals in the inflamed site *via* insufficient production of IL-6 and induction of IL-10 generation which results in the reduced recruitment of effector myeloid cells.

We also studied the effect of ammonium metavanadate and potassium dichromate on the cellular response in rats with aseptic inflammation. Expansion and activation of effector T cell subsets during the inflammatory response represent a normal physiological process directed at the localization of inflammation (Pennock *et al*. 2013). An effect of chromium and vanadium-containing compounds on T cell numbers and activation was described by a limited number of studies. Cobalt-chromium-molybdenum disc samples inhibited proliferation of human peripheral blood T lymphocytes *in vitro* (Wang *et al*. 1996). Dietary ammonium metavanadate inhibited the proliferative activity of $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ splenic T cells and depressed their activity in broilers (Cui *et al*. 2011). It was proposed that vanadium affects migration of mature T cells from the thymus to the spleen (Tsave *et al*. 2016). A modified thymus cortex–medulla ratio (Ustarroz-Cano *et al*. 2017) and a decrease in thymic CD11c dendritic cells, which are necessary for T cells maturation, were observed in mice exposed to vanadium by inhalation (Lee *et al*. 2001). In our experiments, exposure to ammonium metavanadate and potassium dichromate inhibited IFNγ and IL-4 production by $CD4^+$ cells triggered by ongoing aseptic inflammation. The mechanisms underlying the suppression of IFNγ and IL-4 production may involve modulation of nuclear factor of activated T cells (NFAT) and NF-κB activity. After T cell activation, the p50 and p65 NF-κB subunits and NFAT protein bind specifically to the newly identified IFN-γ κB and C3-related sites enhancing the transcriptional activity of the IFN-γ promoter (Sica *et al*. 1997). NF-κB is also involved in the molecular mechanisms controlling IL-4 gene transcription activation (Oh and Ghosh 2013). It was previously shown that exposure to orthometavanadate inhibited activation of NFAT, but enhanced inducible forms of cAMP response element-binding protein (CREB) and activation of the p50/p65 heterodimeric form of NF-κB in both resting and antigen-stimulated T cells (Ortega-Gómez *et al*. 2013). On the other hand, hexavalent chromium was shown to inhibit NF-κB transcription decreasing the interaction of p65 subunit of NF-κB and the transcriptional cofactor cAMP-responsive element-binding protein-binding protein (Shumilla *et al*. 1999). Taking into account that IFNγ and IL-4 are well-known inducers of cell-mediated and humoral immune responses, correspondingly (Dobrakowski *et al*. 2016, Yang *et al*. 2017), we can assume that exposure to ammonium metavanadate and potassium dichromate is able to inhibit both types of immune responses.

In summary, here we show that dietary ammonium metavanadate and potassium dichromate are able to distort the normal course of acute inflammation *via* shifting the pro-inflammatory signals to activation of an anti-inflammatory cytokine IL-10, inhibition of neutrophil expansion and $CD4^+$ T cell activation, which together can prevent the resolution of inflammation and, therefore, favor the pathogenesis of chronic inflammatory processes.

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Disclosure statement

The authors declare that they have no competing interests.

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