

World Journal of *Clinical Cases*

World J Clin Cases 2020 January 6; 8(1): 1-244



REVIEW

- 1 Role of oxysterol-binding protein-related proteins in malignant human tumours
Liu H, Huang S

ORIGINAL ARTICLE**Case Control Study**

- 11 Oncogenic role of Tc17 cells in cervical cancer development
Zhang ZS, Gu Y, Liu BG, Tang H, Hua Y, Wang J

Retrospective Study

- 20 Acute distal common bile duct angle is risk factor for post-endoscopic retrograde cholangiopancreatography pancreatitis in beginner endoscopist
Han SY, Kim DU, Lee MW, Park YJ, Baek DH, Kim GH, Song GA
- 29 Three-dimensional computed tomography mapping of posterior malleolar fractures
Su QH, Liu J, Zhang Y, Tan J, Yan MJ, Zhu K, Zhang J, Li C
- 38 Application of a modified surgical position in anterior approach for total cervical artificial disc replacement
Hou WX, Zhang HX, Wang X, Yang HL, Luan XR
- 46 Potential role of the compound Eucommia bone tonic granules in patients with osteoarthritis and osteonecrosis: A retrospective study
Hu CX, Hu KY, Wang JF
- 54 Prognostic factors for overall survival in prostate cancer patients with different site-specific visceral metastases: A study of 1358 patients
Cui PF, Cong XF, Gao F, Yin JX, Niu ZR, Zhao SC, Liu ZL
- 68 Application of multiple Roux-en-Y hepaticojejunostomy reconstruction by formation of bile hilar duct lake in the operation of hilar cholangiocarcinoma
Yang XJ, Dong XH, Chen SY, Wu B, He Y, Dong BL, Ma BQ, Gao P
- Observational Study**
- 76 Relationship between β -amyloid protein 1-42, thyroid hormone levels and the risk of cognitive impairment after ischemic stroke
Mao L, Chen XH, Zhuang JH, Li P, Xu YX, Zhao YC, Ma YJ, He B, Yin Y

Prospective Study

- 88 Can the wet suction technique change the efficacy of endoscopic ultrasound-guided fine-needle aspiration for diagnosing autoimmune pancreatitis type 1? A prospective single-arm study
Sugimoto M, Takagi T, Suzuki R, Konno N, Asama H, Sato Y, Irie H, Watanabe K, Nakamura J, Kikuchi H, Takasumi M, Hashimoto M, Kato T, Hikichi T, Notohara K, Ohira H

CASE REPORT

- 97 Pembrolizumab - emerging treatment of pulmonary sarcomatoid carcinoma: A case report
Cimpeanu E, Ahmed J, Zafar W, DeMarinis A, Bardarov SS, Salman S, Bloomfield D
- 103 Sclerosing angiomatoid nodular transformation of the spleen, a rare cause for splenectomy: Two case reports
Chikhladze S, Lederer AK, Fichtner-Feigl S, Wittel UA, Werner M, Aumann K
- 110 Postpartum pubic symphysis diastasis-conservative and surgical treatment methods, incidence of complications: Two case reports and a review of the literature
Norvilaite K, Kezeviciute M, Ramasauskaite D, Arlauskiene A, Bartkeviciene D, Uvarovas V
- 120 Use of omental patch and endoscopic closure technique as an alternative to surgery after endoscopic full thickness resection of gastric intestinal stromal tumors: A series of cases
Sachdev AH, Iqbal S, Ribeiro IB, de Moura DTH
- 126 Primary maxillary chondrosarcoma: A case report
Cuevas-González JC, Reyes-Escalera JO, González JL, Sánchez-Romero C, Espinosa-Cristóbal LF, Reyes-López SY, Tovar Carrillo KL, Donohue Cornejo A
- 133 Hyalinizing clear cell carcinoma-a rare entity in the oral cavity: A case report
Donohue-Cornejo A, Paes de Almeida O, Sánchez-Romero C, Espinosa-Cristóbal LF, Reyes-López SY, Cuevas-González JC
- 140 Jejunal cavernous lymphangioma manifested as gastrointestinal bleeding with hypogammaglobulinemia in adult: A case report and literature review
Tan B, Zhang SY, Wang YN, Li Y, Shi XH, Qian JM
- 149 Large pelvic mass arising from the cervical stump: A case report
Zhang K, Jiang JH, Hu JL, Liu YL, Zhang XH, Wang YM, Xue FX
- 157 Mechanical intestinal obstruction due to isolated diffuse venous malformations in the gastrointestinal tract: A case report and review of literature
Li HB, Lv JF, Lu N, Lv ZS
- 168 Two-level percutaneous endoscopic lumbar discectomy for highly migrated upper lumbar disc herniation: A case report
Wu XB, Li ZH, Yang YF, Gu X

- 175 Successful treatment of congenital palate perforation: A case report
Zhang JF, Zhang WB
- 179 Calcitonin-negative neuroendocrine tumor of the thyroid with metastasis to liver-rare presentation of an unusual tumor: A case report and review of literature
Cai HJ, Wang H, Cao N, Huang B, Kong FL, Lu LR, Huang YY, Wang W
- 188 Giant exophytic cystic adenomyosis with a levonorgestrel containing intrauterine device out of the uterine cavity after uterine myomectomy: A case report
Zhou Y, Chen ZY, Zhang XM
- 194 Unusual presentation of bladder neuroblastoma in a child: A case report
Cai JB, Wang JH, He M, Wang FL, Xiong JN, Mao JQ, Li MJ, Zhu K, Liang JW
- 200 Value of dynamic plasma cell-free DNA monitoring in septic shock syndrome: A case report
Liu JP, Zhang SC, Pan SY
- 208 Sarcomatoid intrahepatic cholangiocarcinoma mimicking liver abscess: A case report
Wang Y, Ming JL, Ren XY, Qiu L, Zhou LJ, Yang SD, Fang XM
- 217 Clinical characteristics on manifestation and gene mutation of a transient neonatal cyanosis: A case report
Yuan J, Zhu XP
- 222 Six families with balanced chromosome translocation associated with reproductive risks in Hainan Province: Case reports and review of the literature
Chen YC, Huang XN, Kong CY, Hu JD
- 234 Primary intestinal extranodal natural killer/T-cell lymphoma, nasal type: A case report
Dong BL, Dong XH, Zhao HQ, Gao P, Yang XJ

LETTER TO THE EDITOR

- 242 Cluster headache as a manifestation of a stroke-like episode in a carrier of the *MT-ND3* variant m.10158T>C
Finsterer J

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Cases*, Maddalena Zippi, MD, PhD, Doctor, Unit of Gastroenterology and Digestive Endoscopy, Sandro Pertini Hospital, Rome 00157, Italy

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Cases (WJCC, World J Clin Cases)* is to provide scholars and readers from various fields of clinical medicine with a platform to publish high-quality clinical research articles and communicate their research findings online.

WJCC mainly publishes articles reporting research results and findings obtained in the field of clinical medicine and covering a wide range of topics, including case control studies, retrospective cohort studies, retrospective studies, clinical trials studies, observational studies, prospective studies, randomized controlled trials, randomized clinical trials, systematic reviews, meta-analysis, and case reports.

INDEXING/ABSTRACTING

The *WJCC* is now indexed in PubMed, PubMed Central, Science Citation Index Expanded (also known as SciSearch®), and Journal Citation Reports/Science Edition. The 2019 Edition of Journal Citation Reports cites the 2018 impact factor for *WJCC* as 1.153 (5-year impact factor: N/A), ranking *WJCC* as 99 among 160 journals in Medicine, General and Internal (quartile in category Q3).

RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Yan-Xia Xing*
 Proofing Production Department Director: *Yun-Xiaojuan Wu*

NAME OF JOURNAL

World Journal of Clinical Cases

ISSN

ISSN 2307-8960 (online)

LAUNCH DATE

April 16, 2013

FREQUENCY

Semimonthly

EDITORS-IN-CHIEF

Dennis A Bloomfield, Bao-Gan Peng, Sandro Vento

EDITORIAL BOARD MEMBERS

<https://www.wjnet.com/2307-8960/editorialboard.htm>

EDITORIAL OFFICE

Jin-Lei Wang, Director

PUBLICATION DATE

January 6, 2020

COPYRIGHT

© 2020 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS

<https://www.wjnet.com/bpg/gerinfo/204>

GUIDELINES FOR ETHICS DOCUMENTS

<https://www.wjnet.com/bpg/GerInfo/287>

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

<https://www.wjnet.com/bpg/gerinfo/240>

PUBLICATION MISCONDUCT

<https://www.wjnet.com/bpg/gerinfo/208>

ARTICLE PROCESSING CHARGE

<https://www.wjnet.com/bpg/gerinfo/242>

STEPS FOR SUBMITTING MANUSCRIPTS

<https://www.wjnet.com/bpg/GerInfo/239>

ONLINE SUBMISSION

<https://www.f6publishing.com>

Case Control Study

Oncogenic role of Tc17 cells in cervical cancer development

Zun-Sheng Zhang, Ying Gu, Bing-Gang Liu, Hong Tang, Yu Hua, Jun Wang

ORCID number: Zun-Sheng Zhang (0000-0002-9013-5536); Ying Gu (0000-0002-6499-6738); Bing-Gang Liu (0000-0001-8927-5200); Hong Tang (0000-0002-5933-8909); Yu Hua (0000-0002-8230-2920); Jun Wang (0000-0002-3726-3125).

Author contributions: Zhang ZS and Gu Y designed the research; Tang H and Hua Y performed the research; Liu BG analyzed the data; Wang J wrote the paper.

Institutional review board

statement: This study was reviewed and approved by the Shanghai Seventh People's Hospital Ethics Committee.

Informed consent statement: All patients in our study provided informed consent.

Conflict-of-interest statement: The authors declare no conflict of interest.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works

Zun-Sheng Zhang, Ying Gu, Bing-Gang Liu, Hong Tang, Yu Hua, Jun Wang, Department of Obstetrics and Gynecology, Shanghai Seventh People's Hospital, Shanghai 200120, China

Corresponding author: Jun Wang, MSc, Attending Doctor, Department of Obstetrics and Gynecology, Shanghai Seventh People's Hospital, No. 358, Datong Road, Gaoqiao Town, Pudong New Area, Shanghai 200120, China. wanchuang3069629@163.com

Abstract**BACKGROUND**

As one of the subsets of CD8⁺ T cells, Tc17 cells have recently been identified and are characterized by the secretion of interleukin (IL)-17, which is related to inflammatory diseases.

AIM

To assess the status of Tc17 cells in cervical cancer and investigate the biological function of Tc17 cells in cervical cancer development.

METHODS

Flow cytometry assay, immunohistochemistry, and immunofluorescence were performed to detect the levels and phenotype of Tc17 cells in blood and tumor samples from patients with cervical cancer. Prior to cell suspension culture, ELISA was carried out to measure the production of IL-6, IL-1 β , IL-23, CXCL12, and IL-17 in tumor tissue supernatant and co-cultured supernatant of patients with cervical cancer. In addition, multivariate analysis was performed to identify factors associated with overall survival using the Cox proportional hazards model.

RESULTS

Compared with normal tissues, Tc17 cells specifically accumulated in tumor tissues of cervical cancer patients. Cancer cells produced a greater amount of IL-6, IL-1 β , and IL-23, which in turn promoted Tc17 cell polarization. Unlike the traditional cytotoxic CD8⁺ T cells, Tc17 cells secreted IL-17, which subsequently promoted CXCL12 expression in tumor cells, eventually enhancing the proliferation and migration of tumor cells. Thus, the ratio of tumor-infiltrating Tc17 cells was highly correlated with poor clinical outcome in patients with cervical cancer.

CONCLUSION

Our data identified the oncogenic role of Tc17 cells in the development of cervical cancer. We propose that the ratio of Tc17 cells may be a useful index in the prognosis of patients with cervical cancer.

on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Received: October 7, 2019

Peer-review started: October 7, 2019

First decision: November 13, 2019

Revised: November 18, 2019

Accepted: November 30, 2019

Article in press: November 30, 2019

Published online: January 6, 2020

P-Reviewer: Ankathil R, Ortiz-Sanchez E, Rangel-Corona R

S-Editor: Ma YJ

L-Editor: Webster JR

E-Editor: Liu JH



Key words: Cervical cancer; Tc17 cells; Interleukin-17; Cancer development; Biological function; Oncogenic role

©The Author(s) 2020. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Inflammation contributes to cancer development. In this study, it was found that cervical cancer-elicited inflammation increased Tc17-polarizing cytokine production, which attenuated cytotoxic CD8⁺ T cell development. The high level of interleukin-17 production by Tc17 cells led to CXCL12 upregulation and cancer cell migration. Consistent with the oncogenic role of Tc17 cells in cancer development, the ratio of cancer-infiltrating Tc17 cells was highly associated with poor prognosis in patients with cervical cancer. Thus, our data demonstrate that Tc17 cells can be induced in cervical cancers and serve as a meaningful index in the prognosis of patients with cervical cancer.

Citation: Zhang ZS, Gu Y, Liu BG, Tang H, Hua Y, Wang J. Oncogenic role of Tc17 cells in cervical cancer development. *World J Clin Cases* 2020; 8(1): 11-19

URL: <https://www.wjnet.com/2307-8960/full/v8/i1/11.htm>

DOI: <https://dx.doi.org/10.12998/wjcc.v8.i1.11>

INTRODUCTION

As the fourth most common malignant tumor worldwide, the incidence of new cervical cancer is approximately 130000, accounting for 28% of the total number of cases globally. About 20000 women die of cervical cancer each year^[1,2]. Tumor progression has been recognized to be the product of evolving crosstalk between immune cells and tumor cells. By affecting immune cell activation or differentiation, cancer cells escape host immune attack and enhance tumor resistance to immunotherapies^[3,4].

As the primary component of tumor-infiltrating lymphocytes, T cells elicit a crucial effector function in cancer eradication. Recent studies showed that T cells that produce interleukin (IL)-17 are detected in human tumors, which have a certain pro-inflammatory effect^[5]. On the one hand, Th17 cell polarizing factor may induce differentiation and proliferation of Tc17 cells, despite exhibiting reduced cytotoxic activity in CD8⁺ T cells, thereby interrupting host immune surveillance^[5,6]. Moreover, in a monkey immunodeficiency virus infection model with mammals such as macaques and black-and-white marmosets, Tc17 cells play a pathological role in promoting disease progression^[7,8]. However, the role of Tc17 cells in human cervical cancer development remains unclear.

A large number of Tc17 cells were found in black and white monkey tumor tissues, which were induced by the cytokine IL-23^[8]. At the same time, other studies have shown that IL-6 may also be crucial for Tc17 cell differentiation^[9]. However, it is unclear whether other cytokines can induce Tc17 cell differentiation and how Tc17 cells affect cancer development is still poorly understood.

In this study, we showed that Tc17 cells are highly enriched within cervical cancer tissue. Cervical cancer cells produced a large amount of IL-6, IL-1 β , and IL-23, which induced Tc17 cell polarization. Increased levels of IL-17 induced by Tc17 cells led to CXCL12 upregulation in tumor cells, resulting in tumor cell proliferation and migration. Moreover, the percentage of Tc17 cells was associated with tumor progression and clinical outcome in patients with cervical cancer. Our data demonstrate the oncogenic role of Tc17 cells in cancer development and provide a theoretical basis for the clinical treatment of cervical cancer.

MATERIALS AND METHODS

Patients and tissue specimens

Fresh blood, tumor, peritumoral, or matched adjacent tissues (at least 5 cm distant from the tumor site) were obtained from patients with cervical cancer who underwent surgical resection at the Shanghai Seventh People's Hospital. None of these patients had received chemotherapy or radiotherapy before sampling. Individuals with autoimmune disease, infectious diseases, and multi-primary cancers were excluded.

Blood from healthy donors was used for control experiments. The clinical stages of tumors were determined according to the TNM classification system of the International Union Against Cancer.

Isolation and stimulation of tumor cells

Fresh tissues were washed 3 times with Hank's solution containing 1% fetal calf serum (FCS) before being cut into small pieces. The specimens were then placed in RPMI 1640 medium containing 1 mg/mL collagenase IV and 10 mg/mL deoxyribonuclease I and mechanically dissociated using a gentle MACS Dissociator (Miltenyi Biotec, Bergisch Gladbach, Germany). Dissociated cell suspensions were further incubated for 1 h at 37 °C under continuous rotation. The cell suspensions were then filtered through a 70 µm cell strainer (BD Labware, Bedford, MA, United States). Then, part of cells was used for flow cytometry to detect the number of Tc17 cells, and the remaining cells were cultured with IL-17 (0-10 ng/mL), IL-22 (0-10 ng/mL), interferon (IFN)-γ (0-10 ng/mL), IL-17 plus IL-22, or 100% Tc17 cell-polarizing culture for 48 h. The culture supernatants were harvested for ELISA.

In vitro monocyte-T-cell co-culture system

Peripheral blood mononuclear cells from cervical cancer patients were isolated by Ficoll density gradient centrifugation. Fresh peripheral blood CD8⁺ T cells were selected using positive isolation and negative isolation kits, respectively. In a 5-d incubation period, bead-puriWed peripheral CD8⁺ T cells were co-cultured with autologous blood monocytes at a 2:1 ratio in the presence or absence of recombinant human IL-6 (10 ng/mL), IL-1β (10 ng/mL) and IL-23 (10 ng/mL) in 200 µL RPMI 1640 medium supplemented with 10% FCS. After 5-d incubation, the supernatants were harvested for ELISA and the cells for intracellular cytokine staining.

Flow cytometry analysis

For detection of intracellular molecules, T lymphocytes were stimulated for 5 h with phorbol myristate acetate (50 ng/mL) plus ionomycin (1 µg/mL) in the presence of GolgiStop (BD Pharmingen, San Diego, CA, United States). Intracellular cytokine staining was performed after fixation and permeabilization using Perm/Wash solution (BD Pharmingen). The lymphocytes were analyzed by multicolor flow cytometry with FACSCanto II (BD Biosciences). Data were analyzed with FlowJo software (TreeStar, Ashland, OR, United States) or FACSDiva software (BD Biosciences).

Immunohistochemistry assay

Paraformaldehyde-fixed and paraffin-embedded samples were cut into 5 µm sections, which were incubated with rabbit anti-IL-17 antibody and stained by horseradish peroxidase anti-rabbit immunoglobulin G followed by diaminobenzidine. The sections were then incubated with mouse anti-CD8 antibody and stained using EnVision G2 System/AP Rabbit/Mouse (Permanent Red) (Dako, Glostrup, Denmark) and subsequently counterstained with hematoxylin. Slides were examined using an Olympus 71 inverted fluorescence microscope.

Immunofluorescence

Immunofluorescence was performed as previously described with minor modifications^[10]. In detail, Paraformaldehyde-Waxed cryostat sections of tumor tissues were washed in PBS and blocked for 30 min with 20% rabbit serum in PBS. Sections were incubated with goat anti-human IL-17 antibody (Ab) diluted in 5% rabbit serum. The bound Ab was detected with FITC-conjugated rabbit anti-goat Ab. After washing with PBS, the sections were blocked for 30 min with 20% goat serum in PBS and incubated with mouse anti-human CD8 Ab diluted in 5% goat serum. The bound Ab was detected with TRITC-conjugated goat anti-mouse Ab. After washing with PBS, the slides were examined with an Olympus 71 inverted fluorescence microscope.

ELISA

Cervical cancer tissues or their matched adjacent normal tissues were homogenized in 1 mL Protein Extraction Reagent (Rockford, IL, United States). Concentrations of IL-6, IL-1β, and IL-23 in the tissue supernatants; concentrations of CXCL12 in the co-culture supernatants or tissue supernatants; and concentrations of IL-17 in the co-culture supernatants were determined using ELISA kits according to the manufacturer's instructions.

Statistical analysis

Comparisons between data groups were performed as stated in each figure legend

with the use of GraphPad Prism ver.6. A value of $P < 0.05$ was considered statistically significant. The resulting data were presented as mean \pm SE.

RESULTS

Tc17 cells specifically accumulate in tumor tissues of cervical cancer patients

To assess the status of Tc17 cells in human cervical cancer tissues, we isolated immune cells from cancer tissues, matched adjacent normal tissues as well as peripheral blood. Compared with healthy donors, the percentage of Tc17 cells in the peripheral blood from patients with cervical cancers was identical (Figure 1A). Of note, we found that Tc17 cells were selectively induced in tumors compared to their matched adjacent normal tissues (Figure 1A). To further confirm this result, we analyzed the distribution of Tc17 cells in the paracancerous stroma, carcinoma nets, and intra-tumor sites. The results showed that Tc17 cells accumulated in all these sites, especially in carcinoma nets and intra-tumor sites (Figure 1B-D). Thus, these data show that Tc17 cells have an oncogenic role in cervical cancer development.

Cervical cancer cells produce a greater amount of Tc17-polarizing cytokines

Previous studies revealed that IL-6, IL-1 β , and IL-23 are essential for Tc17 cell differentiation^[11,12]. To investigate the mechanism of cervical cancer following modulation of Tc17 cell development, we assessed the levels of Tc17-polarizing cytokines in cancer-associated tissues. As expected, the concentrations of IL-6, IL-1 β , and IL-23 were significantly increased in peritumoral and intra-tumor tissue relative to their matched normal tissues or peripheral blood (Figure 2A-C). Thus, these data indicate that tumor-derived cytokines play a stimulatory role in the modulation of Tc17 polarization.

IL-6, IL-1 β , and IL-23 acts synergistically to enhance Tc17 cell differentiation

To evaluate the potential role of these cytokines in Tc17 cell differentiation, we isolated peripheral blood CD8⁺ T cells and autologous blood monocytes of cervical cancer patients. After 5-day co-culture incubation, the supernatants were harvested for ELISA and the cells for intracellular cytokine staining. The results showed that the addition of exogenous IL-6, IL-1 β , and IL-23 significantly increased the frequency of Tc17 cells either alone or in combination compared with co-culture without any cytokines added (Figure 3A and B). In addition, the ELISA results showed that IL-17 production was consistent with the percentage of Tc17 cells (Figure 3C). These findings showed that IL-6, IL-1 β , and IL-23 act synergistically to induce Tc17 cell polarization *in vitro* and suggest that a similar process might operate *in vivo*.

Tc17 cell-derived IL-17 promotes CXCL12 expression in tumor cells

To investigate the biological function of Tc17 cells in cervical cancer development, we isolated primary tumor cells and stimulated them with various concentrations of IL-17, IL-22, and IFN- γ , and the production of CXCL12 was detected in the culture supernatants by ELISA. As shown in Figure 4A, CXCL12 expression in cancer cells was increased upon IL-17 stimulation, while IL-22 or IFN- γ exhibited little effects on CXCL12 upregulation in cancer cells (Figure 4A). Moreover, unlike the co-culture supernatants from blood or non-tumor tissue monocytes, TAM-derived Tc17 cell culture supernatants selectively induced CXCL12 production in cancer cells (Figure 4B). The effects of CXCL12 upregulation were attenuated by anti-IL-17 neutralizing antibody, but not anti-IL-22 neutralizing antibody (Figure 4B). These findings suggested that Tc17 cell-derived IL-17 induced chemokine CXCL12 production by tumor cells.

Tumor-infiltrating Tc17 cells are related to the poor outcome of patients with cervical cancer

As CXCL12 upregulation drives cancer development, we assessed the relationship between the number of Tc17 cells and the survival rate of patients with cervical cancer. We first analyzed the number of Tc17 cells per million total cells in each tissue from patients in different TNM stages. The results showed that as cancer progressed, the number of Tc17 cells significantly increased in each of the tested tissues (Figure 5A). Conversely, patient survival was significantly reduced (Figure 5A and B). Simultaneously, we analyzed Tc17 cell percentage within the total CD8⁺ T cells in the same samples, and as cancer progressed, we found that the percentage of Tc17 cells significantly increased in the tested tissues, and was negatively associated with patient survival (Figure 5C and D). These findings suggested that the number of Tc17 cells was related to the survival rate of patients, thereby predicting the clinical

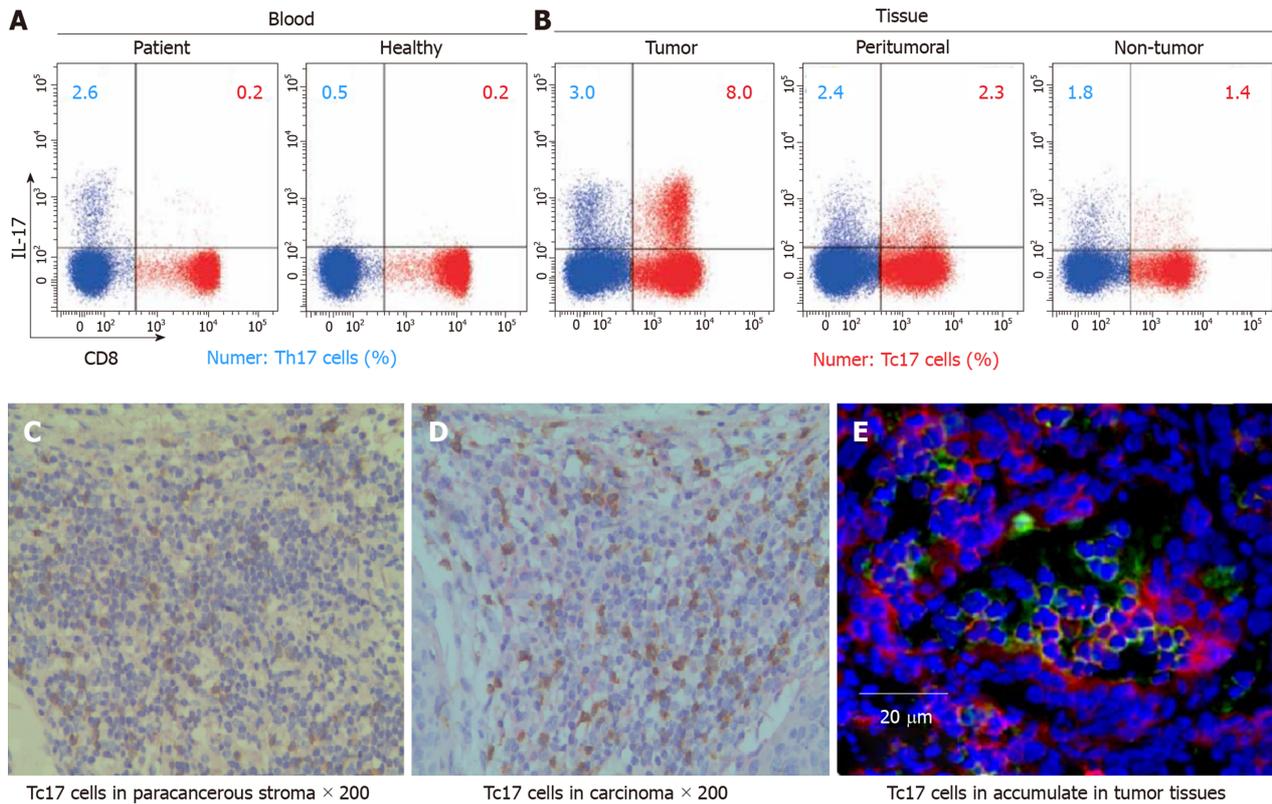


Figure 1 Tc17 cells accumulate in tumor tissues of cervical cancer patients. A, B: Dot plots of intracellular cytokine staining for Tc17 and Th17 in tumor cells; C: Immunohistochemistry staining for interleukin (IL)-17⁺ Tc17 cells in paracancerous stroma, the brown signal represents staining of IL-17, and the red signal represents staining of Tc17 (Envision, ×200); D: Immunohistochemistry staining for IL-17⁺ Tc17 cells in carcinoma nets, the brown signal represents staining of IL-17, and the red signal represents staining of Tc17 (Envision, ×200); E: Immunofluorescence staining for intra-tumoral IL-17⁺ Tc17 cells, the green signal represents staining of IL-17, the red signal represents staining of Tc17, and the blue signal represents DAPI-stained nuclei (scale bar, 20 μm). IL: Interleukin.

outcome of patients with cervical cancer.

DISCUSSION

Although Tc17 cell-mediated immune regulation has been studied in tumor progression in both mice and black-and-white monkeys^[13], the mechanism of Tc17 cells in human cervical cancer has been rarely reported. In this study, we found that Tc17 cells play a stimulatory role in the progression of cervical cancer. Compared with normal tissues, cervical cancer cells produced a greater amount of IL-6, IL-1β, and IL-23A, which are essential for Tc17 cell differentiation^[11,12]. Tc17 induction in turn augmented CXCL12 expression in tumor cells *via* activation of IL-17 signaling. Furthermore, the increased ratio of Tc17 cells in tumors predicted a poor prognosis in patients with cervical cancers. Thus, these data demonstrate that Tc17 cells have an oncogenic role in cervical cancer development.

Recent studies have shown that persistent antigenic stimulation leads to CD8⁺ T cell exhaustion^[14,15]. Blockade of the PD-1/PD-L1 strategy is promising for the treatment of cancer^[16]. However, an increasing number of studies have revealed that tumor-elicited inflammation enhances resistance to cancer immunotherapy, which suggests that cancer cells adopt other strategies to escape immune attack. In the present study, we found that Tc17 cells specifically accumulated in cervical cancer tissue. Unlike the necessary role of IL-6 and IFN-γ in stimulation of Tc17 cell expansion in colorectal or liver cancers^[14,15], cervical cancer cells secrete abundant Tc17-polarizing cytokines including IL-6, IL-1β, and IL-23, suggesting the enhanced effects on Tc17 cell development is attributed to the cancer microenvironment.

Murine tumor models have shown that Tc17 cells impair immune surveillance and promote *de novo* carcinogenesis and neovascularization of tumors^[16,17]. However, other groups have identified anti-tumor activity of Tc17 cells in mice^[18,19]. Therefore, the precise underlying mechanism is still unclear. Our data revealed that increased IL-17 derived from Tc17 cells drives CXCL12 expression in tumor cells, which has been reported to enhance cell proliferation and migration, thereby exacerbating malignant

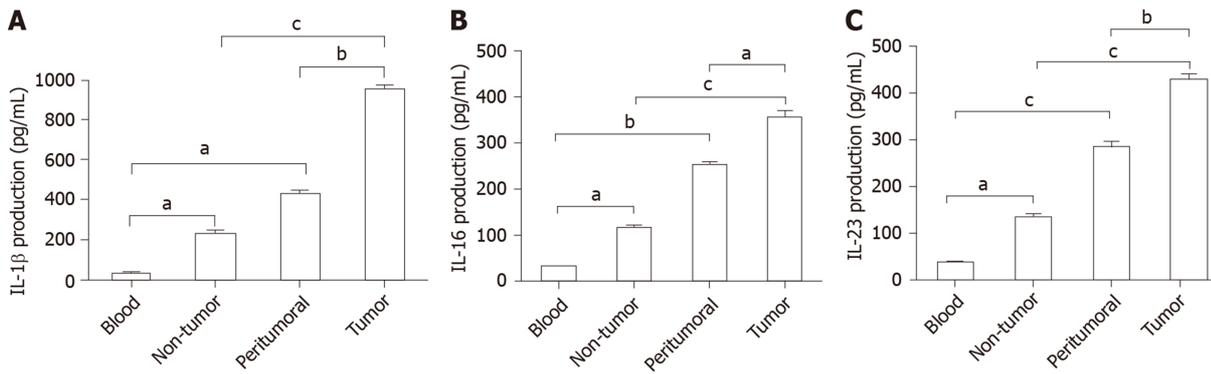


Figure 2 Interleukin-6, interleukin-1β, and interleukin-23 accumulated in tumor tissues of cervical cancer patients. A-C: Before cell suspension culture, ELISA was carried out to show that interleukin (IL)-6, IL-1β, and IL-23 were significantly upregulated in the supernatants of tumor and peritumoral tumor tissues compared with the supernatants isolated from autologous non-tumor tissues or peripheral blood. Comparisons were performed using the *t*-test. ^a*P* < 0.05; ^b*P* < 0.01. ^c*P* < 0.001. Error bars represent SE. IL: Interleukin.

formation. Finally, we assessed the relationship between the number of Tc17 cells and the survival rate of patients with cervical cancer. Similar studies have been conducted in mice and gastric cancer patients^[20]. The results showed that the increased ratio of Tc17 in tumors is highly correlated with poor outcome in patients with cervical cancer.

In conclusion, our data demonstrate that Tc17 cells are specifically induced by the cervical cancer microenvironment. Tc17 cells promote tumor development *via* activation of IL-17 signaling and are associated with the prognosis of patients with cervical cancer. Thus, we propose that Tc17 cells can be used as a useful clinical index in the prognosis of cervical cancer.

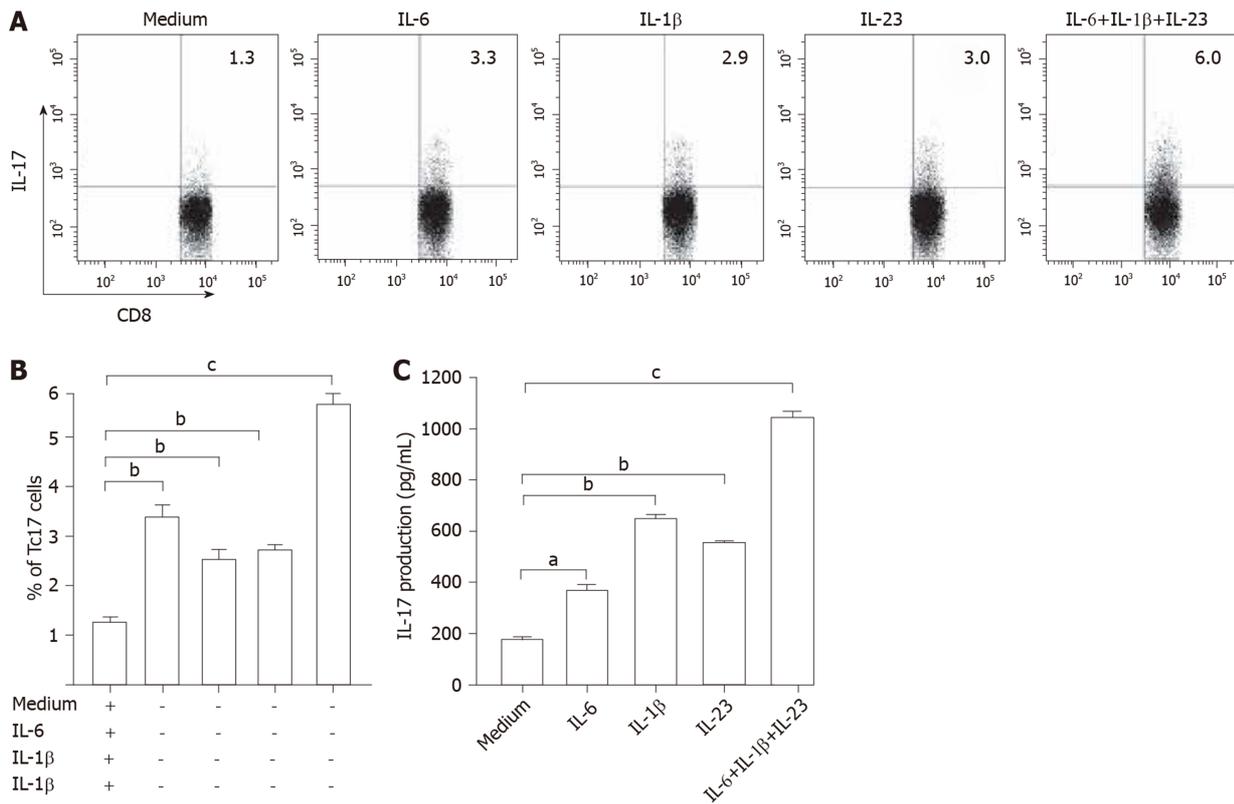


Figure 3 Interleukin-6, interleukin-1 β , and interleukin-23 induced differentiation of Tc17 cells. A-C: Peripheral CD8⁺ T cells and blood monocytes were co-cultured as described in Materials and Methods. Representative data and statistical analysis of Tc17 cell percentage in CD8⁺ T cells and interleukin-17 production in the culture supernatants from the co-culture systems. Comparisons were performed using the *t*-test. ^a*P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001. Error bars represent SE. IL: Interleukin.

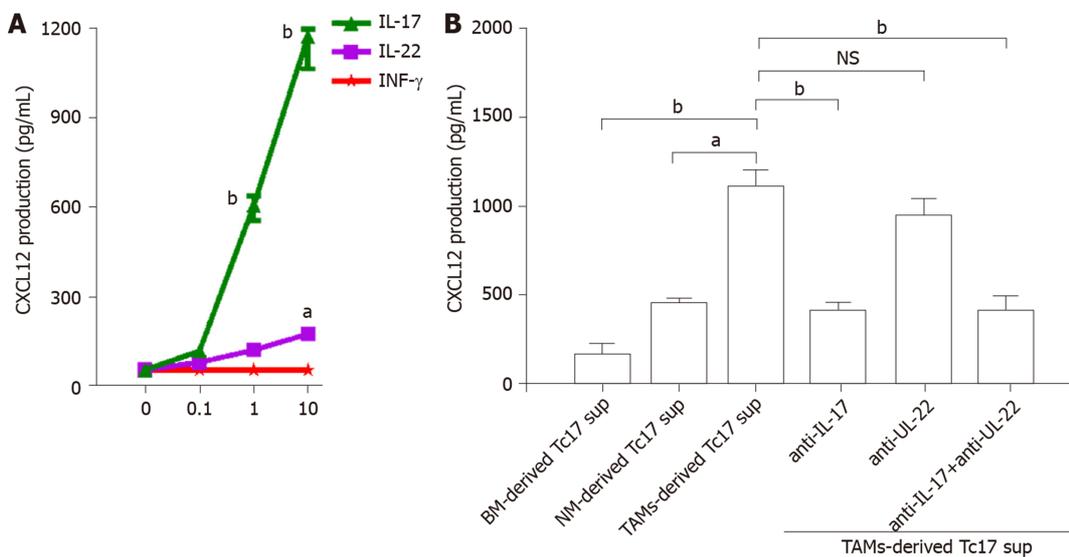


Figure 4 Tc17 cell-derived interleukin-17 induces tumor cells to produce CXCL12. A: Primary tumor cells were cultured with various concentrations of interleukin (IL)-17, IL-22, interferon- γ , and the production of CXCL12 was detected in the culture supernatants by ELISA and statistically analyzed; B: Primary tumor cells were cultured with various concentrations of cytokines or monocyte-derived Tc17 cells culture supernatants, and the production of CXCL12 was detected in the culture supernatants by ELISA and statistically analyzed. Comparisons were performed using the *t*-test. ^a*P* < 0.05; ^b*P* < 0.01. Error bars represent SE. NS: No significant difference; NM: Non-tumor tissue monocyte-derived; BM: Blood monocyte-derived; TAMs: Tumor tissue monocyte-derived.

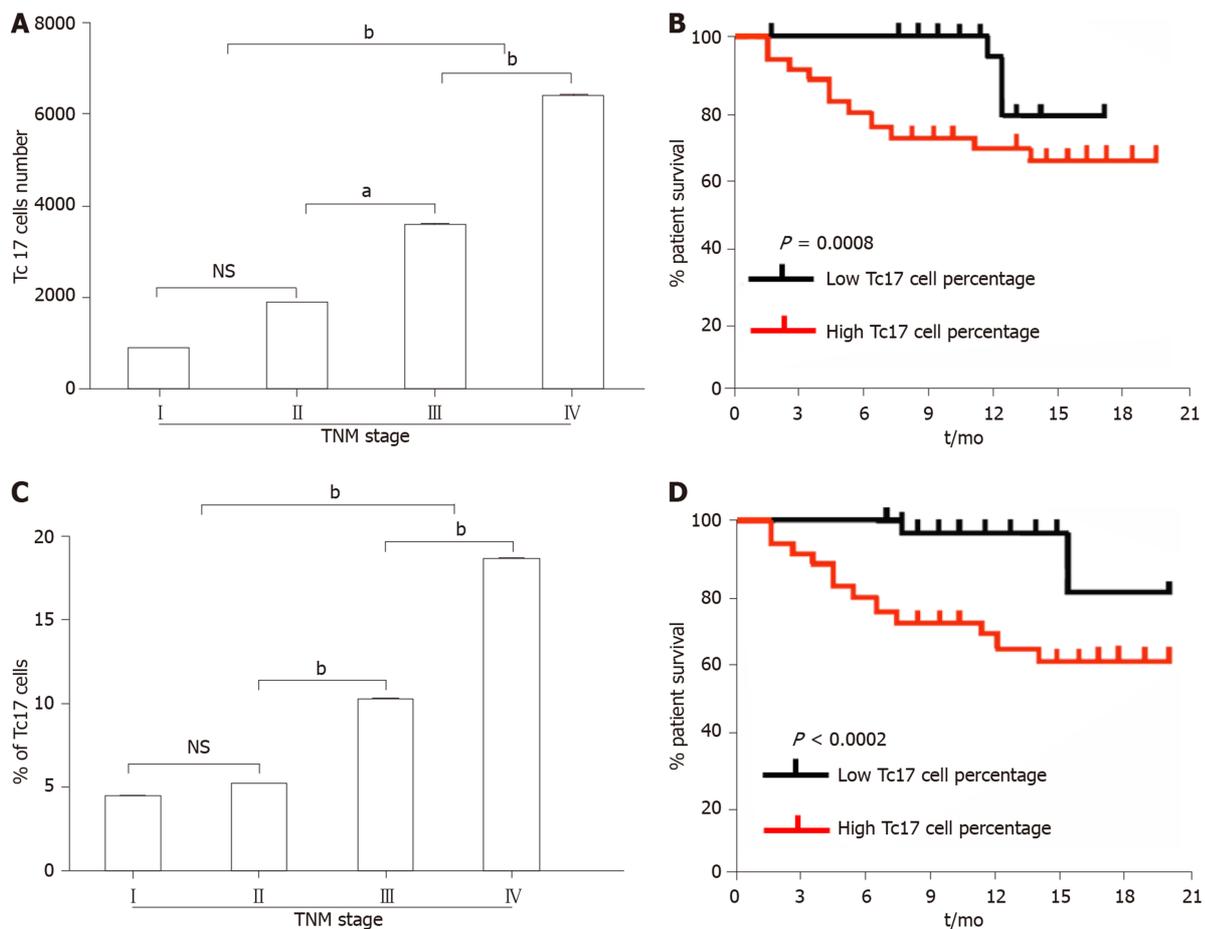


Figure 5 Analysis of the correlation between the percentage of Tc17 cells and the survival rate of cervical cancer patients. A: The total number of Tc17 cells per million total cells in each tissue in different TNM stage; B: The association between Tc17 cell number and patient survival times; C: Tc17 cell percentage in CD8⁺ T cells in each stage of cancer progression; D: Survival significantly decreased as a function of Tc17 cell percentage. Comparisons were performed using the *t*-test. NS indicates no significant difference; ^a*P* < 0.05; ^b*P* < 0.01. Error bars represent SE.

ARTICLE HIGHLIGHTS

Research background

The existence of Tc17 cells was recently shown in several types of inflammatory diseases.

Research motivation

The distribution and functions of Tc17 cells in cervical cancer have not been fully elucidated.

Research objectives

To investigate the role of Tc17 cells in the pathogenesis of cervical cancer.

Research methods

The frequency of Tc17 cells in blood and tumor samples from patients with cervical cancer was determined by flow cytometry. In addition, the levels and phenotype of Tc17 cells in tissue samples from cervical cancer patients were assessed by immunohistochemistry staining.

Research results

Tc17 cells specifically accumulate in the tumor tissues of cervical cancer patients. Cervical cancer-elicited inflammation increases Tc17-polarizing cytokine production, which attenuates cytotoxic CD8⁺ T cell development. High interleukin-17 production by Tc17 cells leads to CXCL12 upregulation and cancer cell migration.

Research conclusions

Consistent with the oncogenic role of Tc17 cells in cancer development, the ratio of cancer-infiltrating Tc17 cells is highly associated with poor prognosis in patients with cervical cancer.

Research perspectives

This study indicates that Tc17 cells in cervical cancer and their regulatory mechanisms are associated with cancer progression.

REFERENCES

- 1 **Chen W**, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115-132 [PMID: 26808342 DOI: 10.3322/caac.21338]
- 2 **Li S**, Hu T, Lv W, Zhou H, Li X, Yang R, Jia Y, Huang K, Chen Z, Wang S, Tang F, Zhang Q, Shen J, Zhou J, Xi L, Deng D, Wang H, Wang S, Xie X, Ma D. Changes in prevalence and clinical characteristics of cervical cancer in the People's Republic of China: a study of 10,012 cases from a nationwide working group. *Oncologist* 2013; **18**: 1101-1107 [PMID: 24043599 DOI: 10.1634/theoncologist.2013-0123]
- 3 **Tu JF**, Pan HY, Ying XH, Lou J, Ji JS, Zou H. Mast Cells Comprise the Major of Interleukin 17-Producing Cells and Predict a Poor Prognosis in Hepatocellular Carcinoma. *Medicine (Baltimore)* 2016; **95**: e3220 [PMID: 27043690 DOI: 10.1097/MD.00000000000003220]
- 4 **Yamaguchi Y**, Seino Y, Takei H, Kurosumi M, Hayashi S. Detection of estrogen-independent growth-stimulating activity in breast cancer tissues: implication for tumor aggressiveness. *Cancer Microenviron* 2014; **7**: 23-31 [PMID: 24203105 DOI: 10.1007/s12307-013-0139-x]
- 5 **Ma J**, Shi LL, Deng YK, Wang H, Cao PP, Long XB, Zhang XH, Liu Y, Zeng M, Liu Z. CD8(+) T cells with distinct cytokine-producing features and low cytotoxic activity in eosinophilic and non-eosinophilic chronic rhinosinusitis with nasal polyps. *Clin Exp Allergy* 2016; **46**: 1162-1175 [PMID: 27176491 DOI: 10.1111/cea.12758]
- 6 **Coughlan L**, Lambe T. Measuring Cellular Immunity to Influenza: Methods of Detection, Applications and Challenges. *Vaccines (Basel)* 2015; **3**: 293-319 [PMID: 26343189 DOI: 10.3390/vaccines3020293]
- 7 **Fujita T**, Burwitz BJ, Chew GM, Reed JS, Pathak R, Seger E, Clayton KL, Rini JM, Ostrowski MA, Ishii N, Kuroda MJ, Hansen SG, Sacha JB, Ndhlovu LC. Expansion of dysfunctional Tim-3-expressing effector memory CD8+ T cells during simian immunodeficiency virus infection in rhesus macaques. *J Immunol* 2014; **193**: 5576-5583 [PMID: 25348621 DOI: 10.4049/jimmunol.1400961]
- 8 **Nanjappa SG**, Hernández-Santos N, Galles K, Wüthrich M, Suresh M, Klein BS. Intrinsic MyD88-Akt1-mTOR Signaling Coordinates Disparate Tc17 and Tc1 Responses during Vaccine Immunity against Fungal Pneumonia. *PLoS Pathog* 2015; **11**: e1005161 [PMID: 26367276 DOI: 10.1371/journal.ppat.1005161]
- 9 **Mei Z**, Zhou L, Zhu Y, Jie K, Fan D, Chen J, Liu X, Jiang L, Jia Q, Li W. Interleukin-22 promotes papillary thyroid cancer cell migration and invasion through microRNA-595/Sox17 axis. *Tumour Biol* 2016; **37**: 11753-11762 [PMID: 27022736 DOI: 10.1007/s13277-016-5030-1]
- 10 **Li M**, Pang Z, Xiao W, Liu X, Zhang Y, Yu D, Yang M, Yang Y, Hu J, Luo K. A transcriptome analysis suggests apoptosis-related signaling pathways in hemocytes of *Spodoptera litura* after parasitization by *Microplitis bicoloratus*. *PLoS One* 2014; **9**: e110967 [PMID: 25350281 DOI: 10.1371/journal.pone.0110967]
- 11 **Kimura A**, Naka T, Kishimoto T. IL-6-dependent and -independent pathways in the development of interleukin 17-producing T helper cells. *Proc Natl Acad Sci U S A* 2007; **104**: 12099-12104 [PMID: 17623780 DOI: 10.1073/pnas.0705268104]
- 12 **Nigam P**, Kwa S, Velu V, Amara RR. Loss of IL-17-producing CD8 T cells during late chronic stage of pathogenic simian immunodeficiency virus infection. *J Immunol* 2011; **186**: 745-753 [PMID: 21148794 DOI: 10.4049/jimmunol.1002807]
- 13 **Alvarez Arias DA**, Kim HJ, Zhou P, Holderried TA, Wang X, Dranoff G, Cantor H. Disruption of CD8+ Treg activity results in expansion of T follicular helper cells and enhanced antitumor immunity. *Cancer Immunol Res* 2014; **2**: 207-216 [PMID: 24778317 DOI: 10.1158/2326-6066.CIR-13-0121]
- 14 **In TSH**, Trotman-Grant A, Fahl S, Chen ELY, Zarin P, Moore AJ, Wiest DL, Zúñiga-Pflücker JC, Anderson MK. HEB is required for the specification of fetal IL-17-producing $\gamma\delta$ T cells. *Nat Commun* 2017; **8**: 2004 [PMID: 29222418 DOI: 10.1038/s41467-017-02225-5]
- 15 **Khmaladze Ia**, Kelkka T, Guerard S, Wing K, Pizzolla A, Saxena A, Lundqvist K, Holmdahl M, Nandakumar KS, Holmdahl R. Mannan induces ROS-regulated, IL-17A-dependent psoriasis arthritis-like disease in mice. *Proc Natl Acad Sci USA* 2014; **111**: E3669-E3678 [PMID: 25136095 DOI: 10.1073/pnas.1405798111]
- 16 **Werthmüller N**, Frey B, Rückert M, Lotter M, Fietkau R, Gaipl US. Combination of ionising radiation with hyperthermia increases the immunogenic potential of B16-F10 melanoma cells in vitro and in vivo. *Int J Hyperthermia* 2016; **32**: 23-30 [PMID: 26754406 DOI: 10.3109/02656736.2015.1106011]
- 17 **Tosello Boari J**, Acosta Rodriguez EV. IL-1 β /CD14 pathway induces IL-17 production: Dendritic cells activated with IL-1 β set Th17 cells on fire by CD14-mediated mechanisms. *Immunol Cell Biol* 2016; **94**: 903-904 [PMID: 27725667 DOI: 10.1038/icb.2016.87]
- 18 **Li YX**, Zhang L, Simayi D, Zhang N, Tao L, Yang L, Zhao J, Chen YZ, Li F, Zhang WJ. Human papillomavirus infection correlates with inflammatory Stat3 signaling activity and IL-17 level in patients with colorectal cancer. *PLoS One* 2015; **10**: e0118391 [PMID: 25706309 DOI: 10.1371/journal.pone.0118391]
- 19 **Li A**, Gan Y, Wang R, Liu Y, Ma T, Huang M, Cui X. IL-22 Up-Regulates β -Defensin-2 Expression in Human Alveolar Epithelium via STAT3 but Not NF- κ B Signaling Pathway. *Inflammation* 2015; **38**: 1191-1200 [PMID: 25510212 DOI: 10.1007/s10753-014-0083-z]
- 20 **Shimada K**, Uzui H, Ueda T, Lee JD, Kishimoto C. N-Acetylcysteine Ameliorates Experimental Autoimmune Myocarditis in Rats via Nitric Oxide. *J Cardiovasc Pharmacol Ther* 2015; **20**: 203-210 [PMID: 25147347 DOI: 10.1177/1074248414547574]



Published By Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-2238242
E-mail: bpgoffice@wjgnet.com
Help Desk: <https://www.f6publishing.com/helpdesk>
<https://www.wjgnet.com>

