## Title

Cytokine modulation correlates with severity of monkeypox disease in humans



## Authors

Johnston, Sara C
Johnson, Joshua C
Stonier, Spencer W
et al.

## Publication Date

2015-02-01

## DOI

10.1016/j.jcv.2014.12.001

Peer reviewed

Elsevier has created a Monkeypox Information Center in response to the declared public health emergency of international concern, with free information in English on the monkeypox virus. The Monkeypox Information Center is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its monkeypox related research that is available on the Monkeypox Information Center - including this research content - immediately available in publicly funded repositories, with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the Monkeypox Information Center remains active.

Journal of Clinical Virology 63 (2015) 42–45

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short Communication

# Cytokine modulation correlates with severity of monkeypox disease in humans

Sara C. Johnston[a], Joshua C. Johnson[a,1], Spencer W. Stonier[a], Kenny L. Lin[a], Neville K. Kisalu[b,2], Lisa E. Hensley[a,1], Anne W. Rimoin[c,*]

[a] Virology Division, United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, MD 21702, USA
[b] Department of Microbiology, Immunology, and Molecular Genetics, UCLA, 609 Charles E. Young Dr. East, 1602 Molecular Science Building, Los Angeles, CA 90095, USA
[c] Department of Epidemiology, UCLA School of Public Health, 650 Charles E. Young Dr. South, Los Angeles, CA 90024, USA

ARTICLE INFO

*Article history:*
Received 24 September 2014
Received in revised form 1 December 2014
Accepted 2 December 2014

*Keywords:*
Orthopoxvirus
Monkeypox
Cytokine
Cytokine storm
Regulatory T cell

ABSTRACT

*Background:* Human monkeypox is a zoonotic disease endemic to parts of Africa. Similar to other orthopoxviruses, virus and host have considerable interactions through immunomodulation. These interactions likely drive the establishment of a productive infection and disease progression, resulting in the range of disease presentations and case fatality rates observed for members of the *Orthopoxvirus* genus.
*Objectives:* Much of our understanding about the immune response to orthopoxvirus infection comes from either *in vitro* or *in vivo* studies performed in small animals or non-human primates. Here, we conducted a detailed assessment of cytokine responses to monkeypox virus using serum from acutely ill humans collected during monkeypox active disease surveillance (2005–2007) in the Democratic Republic of the Congo.
*Study design:* Nineteen serum samples that were from patients with confirmed monkeypox virus infections were selected for cytokine profiling. Cytokine profiling was performed on the Bio-Rad Bioplex 100 system using a 30-plex human cytokine panel.
*Results:* Cytokine profiling revealed elevated cytokine concentrations in all samples. Overproduction of certain cytokines (interleukin [IL]-2R, IL-10, and granulocyte macrophage-colony stimulating factor were observed in patients with serious disease (defined as >250 lesions based on the World Health Organization scoring system).
*Conclusions:* The data suggest that cytokine modulation affects monkeypox disease severity in humans.


## 1. Background

Monkeypox virus (MPXV) is an orthopoxvirus similar to smallpox, and can cause an acute systemic lesional disease associated with high morbidity. Over the last 30 years there has been a marked increase in the number of cases reported in the Democratic Republic of the Congo (DRC) [1].

Immunopathogenesis is believed to play a major role in disease severity and outcome during orthopoxvirus infections. Cytokine storm during variola virus infection has been suggested in human cases and non-human primate models [2–4]. Induction of a cytokine storm is driven by inflammatory mediators and cellular constituents which lead to overproduction of inflammatory cytokines, resulting in massive inflammation, sepsis and septic shock [5]. To date, the presence of cytokine storm following MPXV infection is predicted but yet to be empirically demonstrated.

## 2. Objectives

To determine if a cytokine storm is associated with MPXV infection, serum samples were collected from individuals presenting with febrile illness and rash during active monkeypox disease

*Abbreviations:* IFN, interferon; IL, interleukin; MIP, macrophage inflammatory protein; MCP-1, monocyte chemoattractant protein-1; MPXV, monkeypox virus; DRC, Democratic Republic of the Congo; GM-CSF, granulocyte macrophage-colony stimulating factor; PCR, polymerase chain reaction; Th1 cells, T helper 1 cells; Th2 cells, T helper 2 cells; TNF, tumor necrosis factor; Treg, regulatory T cells.

* Corresponding author at: 650 Charles E. Young Dr. South, CHS 71-279B, Box 177220, Los Angeles, CA 90095, USA. Tel.: +1 310 825 2096; fax: +1 310 206 6039.
*E-mail address:* arimoin@ucla.edu (A.W. Rimoin).
[1] Present address: Integrated Research Facility, National Institute of Allergy and Infectious Diseases, 8200 Research Plaza, Fort Detrick, MD 21702, USA.
[2] Present address: Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA.

http://dx.doi.org/10.1016/j.jcv.2014.12.001
1386-6532/

**Table 1**
Metadata of 19 patients infected with monkeypox from health zones within the Sankuru District of the Democratic Republic of the Congo.

| Patient ID | Health zone | Age | Sex | Disease severity[a] | Infection source | Monkeypox lineage | Genbank accession # |
|---|---|---|---|---|---|---|---|
| 06MPX0966 | Katako Kombe | 20.0 | M | Mild | Unknown | A | – |
| 06MPX0970 | Katako Kombe | 10.0 | F | Serious | Human | C | JX878408 |
| 06MPX1082 | Katako Kombe | 24.0 | M | Severe | Human | ND | – |
| 06MPX1092 | Kole | 10.0 | F | Mild | Animal | A | – |
| 06MPX1094 | Kole | 1.5 | F | Serious | Human | ND | – |
| 07MPX0013 | Kole | 24.0 | F | Mild | Human | B | – |
| 07MPX0016 | Kole | 2.6 | F | Moderate | Human | B | – |
| 07MPX0019 | Katako Kombe | 9.0 | F | Severe | Human | ND | – |
| 07MPX0021 | Katako Kombe | 13.0 | F | Severe | Human | A | – |
| 07MPX0104 | Bena Dibele | 4.0 | F | Serious | Unknown | D | JX878417 |
| 07MPX0109 | Lodja | 3.0 | M | Serious | Human | ND | – |
| 07MPX0275 | Djalo Ndjeka | 10.0 | F | Moderate | Human | C | JX878419 |
| 07MPX0281 | Djalo Ndjeka | 17.0 | M | Moderate | Animal | C | – |
| 07MPX0286 | Lomela | 20.0 | M | Serious | Animal | A | JX878421 |
| 07MPX0412 | Katako Kombe | 3.8 | F | Moderate | Unknown | C | – |
| 07MPX0435 | Djalo Ndjeka | 1.8 | M | Severe | Human | ND | – |
| 07MPX0438 | Djalo Ndjeka | 10.0 | F | Mild | Human | ND | – |
| 07MPX0450 | Kole | 11.0 | M | Severe | Human | C | JX878426 |
| 07MPX0496 | Katako Kombe | 29.0 | F | Moderate | Human | C | – |

ND = not determined.
[a] Disease severity based on the World Health Organization lesion scoring system.

surveillance in the DRC (2005–2007). Monkeypox infection was confirmed by polymerase chain reaction (PCR) [1].

## 3. Study design

### 3.1. Sample acquisition and processing

Methods for sample acquisition and processing are published elsewhere [1]. In MPXV-infected patients [1], disease severity (mild or <25 lesions, moderate or 25–99 lesions, severe or 100–250 lesions, and serious or >250 lesions) was determined using the World Health Organization scoring system used during smallpox eradication [6,7]. Samples were stratified based on geographic location, disease severity, and transmission source (human or animal). Nineteen cases were selected for cytokine profiling. The breakdown of disease severity was: mild = 4 cases; moderate = 5 cases; severe = 5 cases; serious = 5 cases. Ethical approval was obtained from participating institutions, and informed consent was obtained from patients and parents/guardians.

### 3.2. Human cytokine analysis

Serum cytokines were measured in triplicate using Cytokine Human Magnetic 30-Plex Panel (Life Technologies, Grand Island, NY). Briefly, serum samples were thawed, vortexed, and centrifuged to remove any cryoprecipitants. Pre-mixed, lyophilized stock cytokines were rehydrated and serially diluted 3-fold to produce the standard curve. Fifty microliters of standards, or blanks were assayed per replicate well as per manufacturer's instructions (Life Technologies, Grand Island, NY). Data were acquired on a Bio-Rad Bioplex 100 system (Bio-Rad Laboratories, Hercules, CA), and exported into GraphPad Prism (GraphPad Software, La Jolla, CA) for analysis. Normal human cytokine ranges and averages were obtained from the Bio-Plex Suspension Array System Technical Note 6029 available on the Bio-Rad website (www.bio-rad.com). Statistical significance was determined using unpaired T tests conducted at the 95% confidence level.

## 4. Results

### 4.1. Cytokine responses are predictive of disease severity

Nineteen serum samples (Table 1) from confirmed MPXV infections were analyzed using a multiplex cytokine assay. Although concentrations of interleukin (IL)-2R were similar between mild, moderate, and severe cases, concentrations were significantly higher ($P < 0.05$) for serious cases (Table 2). MIP-1α and MIP-1β concentrations in all cases were elevated, and concentrations of these cytokines were significantly elevated ($P < 0.05$) in mild cases compared to moderate and severe cases. Although all cases had elevations in serum concentrations of IL-1RA, IL-6, and IL-15, moderate cases had significantly lower concentrations of IL-1RA (this difference was not statistically significant [$P > 0.05$]), severe cases had significantly lower concentrations of IL-6 ($P < 0.05$), and moderate and severe cases had lower concentrations of IL-15 compared to other disease categories (this difference was not statistically significant [$P > 0.05$]). IL-10 concentrations were elevated above normal range for all categories of disease severity; however, the difference between serious cases and cases in all other severity categories was statistically significant ($P < 0.05$). IL-10 concentrations were roughly proportional to disease severity. Differences between mild, moderate, and severe were not statistically significant ($P > 0.05$). Granulocyte macrophage-colony stimulating factor (GM-CSF) was noticeably elevated above normal human range only for serious disease cases; concentrations in mild, moderate, and severe disease were not significantly different ($P > 0.05$).

Serum concentrations of IL-1β, IL-1RA, IL-2R, IL-4, IL-5, IL-6, IL-8, IL-13, IL-15, IL-17, MCP-1, and RANTES were consistently elevated across all severity categories. Interferon (IFN)-α, IFN-γ, IL-2, IL-7, IP-10, IL-12p40, MIG, eotaxin, and tumor necrosis factor (TNF)-α concentrations were not significantly elevated for any severity category.

## 5. Discussion

From serological examination of cytokine responses to human MPXV infection, a cytokine storm appears to occur during human monkeypox disease. We also found evidence of a prominent T helper 2 (Th2) response, and a dampened Th1 response, following MPXV infection. Th2-associated cytokines IL-4 (and the closely related IL-13), IL-5, and IL-6 were elevated above normal human range and IL-10 was elevated in serious cases, whereas serum concentrations of Th1-associated cytokines (IL-2, TNF-α, IL-12, IFN-γ) fell within normal range for all severity categories. During MPXV infection, Th2 immune responses (*e.g.*, IL-10, IL-4) could downregulate Th1-immune responses (*e.g.*, IL-12, INF-γ, IL-2) as has been seen during infection with recombinant vaccinia virus expressing IL-4 [8]. IL-4, IL-10, and IL-13 increase vaccinia virus replication

**Table 2**
Serum concentrations of cytokines/chemokines in patients with monkeypox infection.

| Serum cytokine/chemokine/growth factor concentrations (pg/ml) | | | | | |
|---|---|---|---|---|---|
| Cytokines, chemokines, or growth factors | Normal range[a] | Mild disease[b] (SEM) | Moderate disease[b] (SEM) | Severe disease[b] (SEM) | Serious disease[b] (SEM) |
| IL-1β | 0.02–0.70 | 134.77 (43.28) | 649.17 (314.10) | 1002.01 (468.50) | 144.82 (67.79) |
| IL-1RA | 0.20–665.00 | 7586.08 (2046.00) | 2656.27 (502.60) | 7480.04 (2946.00) | 6983.32 (2108.00) |
| IL-2 | 0.03–90.00 | 36.08 (9.19) | 4.31 (1.97) | 55.42 (27.49) | 71.02 (37.18) |
| IL-2R | 28.00–594.00 | 1327.69 (186.70) | 1806.10 (154.80) | 1557.05 (168.80) | 4239.94 (401.60)[c] |
| IL-4 | 0.01–3.00 | 170.94 (40.59) | 298.23 (8.44) | 206.17 (28.35) | 258.27 (27.28) |
| IL-5 | 0.01–7.00 | 13.25 (1.02) | 15.07 (0.61) | 16.12 (0.44) | 13.75 (0.50) |
| IL-6 | 0.02–9.00 | 314.09 (84.87) | 795.90 (374.30) | 23.12 (7.10)[c] | 200.82 (48.67) |
| IL-7 | 0.01–14.00 | 25.88 (8.45) | 0.00 (0.00) | 8.63 (3.79) | 8.45 (4.55) |
| IL-8 | 0.08–116.00 | 13,934.80 (2767.00) | 11,242.30 (3892.00) | 10,232.60 (3613.00) | 7944.93 (2736.00) |
| IL-10 | 5.90–637.00 | 5.07 (1.39) | 9.23 (1.04) | 24.54 (9.54) | 377.05 (171.60)[c] |
| IL-12p40 | 36.00–646.00 | 562.22 (116.10) | 692.93 (16.98) | 512.59 (70.32) | 798.86 (90.67) |
| IL-13 | 0.01–9.00 | 24.83 (3.41) | 25.64 (3.33) | 33.24 (2.73) | 26.05 (3.42) |
| IL-15 | 0.06–5.00 | 198.03 (83.50) | 32.31 (15.93) | 53.08 (10.18) | 190.49 (95.07) |
| IL-17 | 0.22–31.00 | 97.40 (19.42) | 100.01 (6.61) | 93.12 (8.76) | 89.04 (9.79) |
| IFN-α | 3.30–63.00 | 87.06 (17.14) | 55.80 (4.96) | 54.12 (4.65) | 60.09 (7.29) |
| IFN-γ | 0.60–124.00 | 86.42 (21.16) | 145.54 (5.20) | 112.31 (13.96) | 135.50 (15.28) |
| TNF-α | 0.10–98.00 | 16.03 (3.71) | 35.62 (8.12) | 17.41 (1.93) | 22.15 (2.96) |
| GM-CSF | 0.80–122.00 | 8.86 (3.41) | 89.62 (44.43) | 101.71 (32.39) | 839.00 (421.90)[c] |
| Eotaxin | 1.20–39.00 | 54.00 (11.51) | 37.35 (4.41) | 24.05 (4.43) | 72.93 (23.15) |
| MCP-1/CCL2 | 2.00–48.00 | 4128.88 (751.00) | 6253.45 (2461.00) | 1514.07 (338.60) | 3621.06 (966.10) |
| MIP-1α/CCL3 | 0.01–2.00 | 480.30 (107.90)[c] | 170.10 (7.51) | 188.17 (26.48) | 323.10 (88.28) |
| MIP-1β/CCL4 | 1.70–47.00 | 899.01 (240.70)[c] | 167.85 (20.04) | 207.37 (20.17) | 354.69 (95.02) |
| RANTES/CCL5 | 100.00–2282.00 | 22,197.50 (10,134.00) | 48,143.00 (12,034.00) | 28,224.70 (9701.00) | 101,828.00 (41,612.00) |
| MIG/CXCL9 | 86.00–7911.00 | 557.48 (108.40) | 370.46 (39.88) | 403.00 (90.98) | 1887.37 (546.70) |
| IP-10/CXCL10 | 5.90–637 | 61.23 (15.69) | 80.05 (13.52) | 66.78 (16.33) | 525.07 (167.00) |

*Abbreviations*: SEM, standard error of the mean; TNF-α, tumor necrosis factor-alpha; IFN, interferon; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; IL-2R, interleukin-2 receptor; IL-12p40, interleukin-12 p40 subunit; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1alpha; RANTES, regulated upon activation normal T cell expressed and presumably secreted; GM-CSF, granulocyte macrophage colony-stimulating factor; SEM, standard error of the mean.

[a] Values were obtained from Technical Note 6029 "Bio-Plex suspension array system" from Bio-Rad (www.bio-rad.com).

[b] Values represent the average for samples belonging to the specific disease category (mild, moderate, severe, serious).

[c] $P < 0.05$ compared to other severity categories.

in patients with atopic dermatitis and in mice deficient in Th1 cytokines challenged with vaccinia virus [8–10]. IL-10 release is often a strong indication of cytokine storm. IL-10 dampens the inflammatory response by downregulating the function of neutrophils, monocytes, and dendritic cells. In addition, IL-10 reduces the production of Th1 cytokines, and promotes the development of regulatory T cells (Treg) and the survival of B cells [11–15]. Although we did not directly assay for a Treg response, the data suggest that further work to elucidate types of T cell responses elicited by MPXV in humans is necessary as these responses may be key contributors to pathogenesis. Alternatively, orthopoxvirus inhibitors of TNF-α, IFN-α, and IFN-γ have been described previously, and our data suggest that immunomodulation of these cytokines might occur during human MPXV infections.

We determined that a certain subset of cytokines were associated with disease severity. MIP-1α and MIP-1β were significantly elevated in cases of mild disease compared to moderate and severe cases. MIPs are pro-inflammatory chemokines which are produced during the innate immune response and promote the migration of granulocytes to areas of infection. GM-CSF, IL-10, and sIL-2R were present in extremely high concentrations in serum samples from serious disease cases. Elevated IL-10, GM-CSF and sIL-2R serum concentrations, in association with tempered IL-2, IFN-γ, and IL-12 responses are commonly indicative of a Treg response and a dampening of the immune response [13,16–23]. Viruses such as HIV and HCV modulate the host immune system to promote the expansion of Tregs, thereby facilitating virus persistence through immune subversion [24]. These data suggest that MPXV may promote similar immune-subversion mechanisms to enable persistence and continued spread, which may result in more serious clinical disease.

Study limitations include time point after exposure that the serum samples were obtained and influence of other microbial agents on immune response to MPXV infection. As serum samples were taken from symptomatic patients (fever, rash) during a surveillance program, duration of infection is unknown but can be assumed to be at least 4 days post-exposure. Also, we cannot rule out the presence of other bacterial, parasitic, or viral agents endemic to the DRC. Despite these limitations, we were able to use cytokine-profiling techniques to assess immunological correlates of monkeypox disease in humans. Our data revealed potential indicators or factors for development of serious disease. Future studies should build on this work to assess these potential biomarkers for serious disease and/or targets for intervention. These data will also be useful in the evaluation of animal models. A major shortcoming for animal models is the lack of human data for comparison. Our study is the first step in compiling human data on immunological correlates of monkeypox disease that can evaluate the relevance of animal models to the human condition.

## Funding

This work was supported by the Defense Threat Reduction Agency [Project # 195726]. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

## Competing interests

None declared.

## Ethical approval

Ethical approval for the original surveillance study was obtained from the participating institutions, and informed consent was obtained from all participants.

## Acknowledgements

Many people assisted with the collection of samples that were used during this study. We would like to specifically thank the Congolese health workers in the Sankuru District for their surveillance efforts and the DRC Ministry of Health and World Health Organization Country office in Kinshasa. We would also like to thank Laura Bollinger (Integrated Research Facility, National Institute of Allergy and Infectious Diseases) for editorial assistance.

## References

[1] Rimoin AW, Mulembakani PM, Johnston SC, Lloyd Smith JO, Kisalu NK, Kinkela TL, et al. Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. Proc Natl Acad Sci U S A 2010;107:16262–7.
[2] Rubins KH, Hensley LE, Jahrling PB, Whitney AR, Geisbert TW, Huggins JW, et al. The host response to smallpox: analysis of the gene expression program in peripheral blood cells in a nonhuman primate model. Proc Natl Acad Sci U S A 2004;101:15190–5.
[3] Jahrling PB, Hensley LE, Martinez MJ, Leduc JW, Rubins KH, Relman DA, et al. Exploring the potential of variola virus infection of cynomolgus macaques as a model for human smallpox. Proc Natl Acad Sci U S A 2004;101:15196–200.
[4] Martin DB. The cause of death in smallpox: an examination of the pathology record. Mil Med 2002;167:546–51.
[5] Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. Microbiol Mol Biol Rev 2012;76:16–32.
[6] Kugelman JR, Johnston SC, Mulembakani PM, Kisalu N, Lee MS, Koroleva G, et al. Genomic variability of monkeypox virus among humans, Democratic Republic of the Congo. Emerg Infect Dis 2014;20:232–9.
[7] Hooper JW, Thompson E, Wilhelmsen C, Zimmerman M, Ichou MA, Steffen SE, et al. Smallpox DNA vaccine protects nonhuman primates against lethal monkeypox. J Virol 2004;78:4433–43.
[8] Sharma DP, Ramsay AJ, Maguire DJ, Rolph MS, Ramshaw IA. Interleukin-4 mediates down regulation of antiviral cytokine expression and cytotoxic T-lymphocyte responses and exacerbates vaccinia virus infection in vivo. J Virol 1996;70:7103–7.
[9] Howell MD, Gallo RL, Boguniewicz M, Jones JF, Wong C, Streib JE, et al. Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus. Immunity 2006;24:341–8.
[10] van Den Broek M, Bachmann MF, Kohler G, Barner M, Escher R, Zinkernagel R, et al. IL-4 and IL-10 antagonize IL-12-mediated protection against acute vaccinia virus infection with a limited role of IFN-gamma and nitric oxide synthetase 2. J Immunol 2000;164:371–8.
[11] Sinuani I, Beberashvili I, Averbukh Z, Sandbank J. Role of IL-10 in the progression of kidney disease. World J Transplant 2013;3:91–8.
[12] Chapoval S, Dasgupta P, Dorsey NJ, Keegan AD. Regulation of the T helper cell type 2 (Th2)/T regulatory cell (Treg) balance by IL-4 and STAT6. J Leukoc Biol 2010;87:1011–8.
[13] Shevach EM. Mechanisms of foxp3+ T regulatory cell-mediated suppression. Immunity 2009;30:636–45.
[14] Akbari O, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH, et al. Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. Nat Med 2002;8:1024–32.
[15] Wurster AL, Rodgers VL, Satoskar AR, Whitters MJ, Young DA, Collins M, et al. Interleukin 21 is a T helper (Th) cell 2 cytokine that specifically inhibits the differentiation of naive Th cells into interferon gamma-producing Th1 cells. J Exp Med 2002;196:969–77.
[16] Kastenmuller W, Gasteiger G, Subramanian N, Sparwasser T, Busch DH, Belkaid Y, et al. Regulatory T cells selectively control CD8+ T cell effector pool size via IL-2 restriction. J Immunol 2011;187:3186–97.
[17] Haeryfar SM, DiPaolo RJ, Tscharke DC, Bennink JR, Yewdell JW. Regulatory T cells suppress CD8+ T cell responses induced by direct priming and cross-priming and moderate immunodominance disparities. J Immunol 2005;174:3344–51.
[18] Ganesh BB, Cheatem DM, Sheng JR, Vasu C, Prabhakar BS. GM-CSF-induced CD11c+CD8a− dendritic cells facilitate Foxp3+ and IL-10+ regulatory T cell expansion resulting in suppression of autoimmune thyroiditis. Int Immunol 2009;21:269–82.
[19] Sheng JR, Li L, Ganesh BB, Vasu C, Prabhakar BS, Meriggioli MN. Suppression of experimental autoimmune myasthenia gravis by granulocyte-macrophage colony-stimulating factor is associated with an expansion of FoxP3+ regulatory T cells. J Immunol 2006;177:5296–306.
[20] Sheng JR, Li LC, Ganesh BB, Prabhakar BS, Meriggioli MN. Regulatory T cells induced by GM-CSF suppress ongoing experimental myasthenia gravis. Clin Immunol 2008;128:172–80.
[21] Pedersen AE, Lauritsen JP. CD25 shedding by human natural occurring CD4+CD25+ regulatory T cells does not inhibit the action of IL-2. Scand J Immunol 2009;70:40–3.
[22] Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J Exp Med 1998;188:287–96.
[23] Pandiyan P, Zheng L, Ishihara S, Reed J, Lenardo MJ. CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. Nat Immunol 2007;8:1353–62.
[24] Mills KH. Regulatory T cells: friend or foe in immunity to infection? Nat Rev Immunol 2004;4:841–55.