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Short Communication

Cytokine modulation correlates with severity of monkeypox disease in humans

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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.

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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