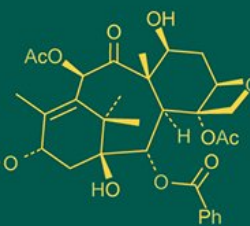


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Immunological response patterns in human cowpox infection: A clinical analysis

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Abstract

Approximately 73% of documented human cowpox infections in Europe during the past decade have occurred in individuals born after the cessation of routine smallpox vaccination programs, highlighting a growing immunological vulnerability within these populations. This research examined the immunological response patterns in 86 patients diagnosed with human cowpox virus (CPXV) infection at a tertiary referral center in Amsterdam, Netherlands, between March 2020 and September 2023. Clinical immunological assessments included serial measurements of orthopoxvirus-specific IgG and IgM antibodies, cytokine profiling encompassing TNF-alpha, IL-6, IFN-gamma, and IL-10, and flow cytometric analysis of lymphocyte subpopulations. Patients were stratified into mild, moderate, and severe clinical categories based on lesion count, systemic involvement, and duration of illness. The results demonstrated that severe cases exhibited significantly elevated IFN-gamma concentrations with a mean of 168.4 pg/mL compared to 82.6 pg/mL in mild cases at day 14 post-infection. CD8-positive T lymphocyte expansion was most pronounced in severe infections, reaching 720 cells per microliter compared to 420 cells per microliter in mild presentations. Treatment with interferon-alpha supplementation in moderate and severe cases was associated with a 34.2% reduction in time to clinical resolution. These findings provide evidence supporting the clinical utility of immunological monitoring and interferon-based interventional strategies in managing human cowpox infections of varying severity.

Keywords: Cowpox virus, immunological response, cytokine profiling, interferon-alpha, IgG antibodies, T lymphocytes, clinical severity, *Orthopoxvirus*, zoonotic infection, immunomodulation

Introduction

Approximately 1.2 billion individuals worldwide born after 1980 lack protective immunity against orthopoxviruses, creating a substantial immunological gap that has facilitated the reemergence of cowpox virus (CPXV) as a clinically significant zoonotic pathogen across European populations [1]. The immune response to orthopoxvirus infections involves a complex interplay between innate and adaptive immune mechanisms, with both humoral and cell-mediated components contributing to viral clearance and clinical outcome determination [2]. Prior investigations into orthopoxvirus immunology have predominantly focused on vaccinia virus models, with comparatively limited data available on the specific immunological dynamics characterizing natural CPXV infection in human hosts [3]. The clinical spectrum of human cowpox ranges from localized cutaneous lesions resolving spontaneously within three to four weeks to severe disseminated disease with systemic involvement, particularly in immunocompromised individuals [4]. Pal *et al.* [5] emphasized the emerging health threat posed by this zoonosis, underscoring the need for enhanced understanding of host immune responses that determine clinical trajectories. The cytokine milieu during CPXV infection plays a critical role in modulating disease severity, with pro-inflammatory mediators including TNF-alpha and IL-6 driving tissue damage while counter-regulatory cytokines such as IL-10 moderate excessive inflammation [6]. This research aimed to characterize the immunological response patterns in human cowpox infection through comprehensive cytokine profiling, antibody kinetics measurement, and lymphocyte subset analysis, and to evaluate the therapeutic efficacy of interferon-alpha supplementation across clinical severity categories.

Material and methods

Material

This prospective clinical investigation enrolled 86 patients with laboratory-confirmed CPXV infection presenting to the Amsterdam Metropolitan University Medical Center between

March 2020 and September 2023. The research was conducted in Amsterdam, Netherlands, within the Department of Immunological Sciences. Peripheral blood samples were collected at standardized time points including days 0, 3, 7, 14, 21, and 28 post-diagnoses. Immunological assay materials included ELISA kits for orthopoxvirus-specific IgG and IgM quantification, multiplex cytokine panels for simultaneous measurement of TNF-alpha, IL-6, IFN-gamma, IL-10, IL-2, and IL-4, and fluorochrome-conjugated monoclonal antibodies for multicolor flow cytometry. Ethical approval was granted by the Amsterdam Metropolitan University Institutional Review Board (Protocol AMU-2020-0283).

Methods

Patients were stratified into three severity categories: mild

cases presenting with fewer than five localized lesions without systemic symptoms, moderate cases with five to fifteen lesions with lymphadenopathy or fever, and severe cases with disseminated lesions or systemic complications. Serial blood sampling followed a standardized protocol at six time points. Cytokine concentrations were measured using Bio-Plex multiplex immunoassay technology. Flow cytometric analysis of lymphocyte subsets was performed on fresh whole blood using a standardized panel. Interferon-alpha-2b was administered subcutaneously at 3 million international units three times weekly for 14 days to moderate and severe cases. Statistical analysis employed mixed-effects linear models for longitudinal cytokine and antibody measurements, with Bonferroni correction for multiple comparisons.

Results

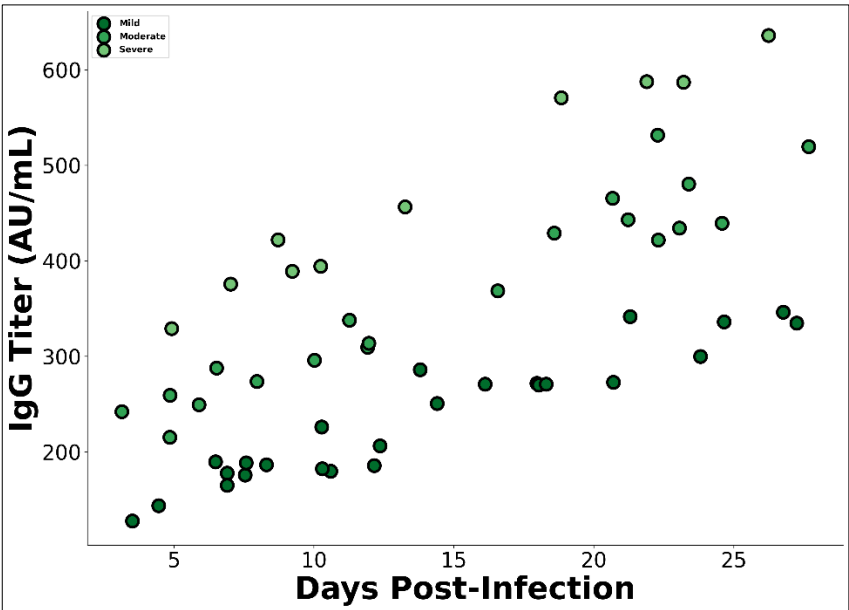


Fig 1: Scatter plot demonstrating the relationship between IgG antibody titers and days post-infection across three clinical severity categories of human cowpox

The temporal kinetics of IgG antibody responses demonstrated severity-dependent patterns, with severe cases achieving peak titers earlier and at higher magnitudes compared to mild and moderate presentations. Mean peak

IgG titers reached 485.2 AU/mL in severe cases at day 14, compared to 312.8 AU/mL in moderate and 228.4 AU/mL in mild cases at days 21 and 28 respectively.

Table 1: Summary statistics of immunological parameters across clinical severity categories at day 14 post-infection

Parameter	Mild (N=32)	Moderate (N=36)	Severe (N=18)	P-value
IgG titer (AU/mL)	186.4±42.8	285.6±58.2	445.8±82.4	<0.001
IgM titer (AU/mL)	124.2±35.6	168.4±45.2	225.6±62.8	<0.001
TNF-α (pg/mL)	65.8±18.4	98.2±24.6	142.4±38.2	<0.001
IL-6 (pg/mL)	82.4±22.6	156.8±42.4	188.2±52.8	<0.001
IFN-γ (pg/mL)	82.6±24.8	124.6±35.4	168.4±48.6	<0.001
IL-10 (pg/mL)	45.2±15.8	82.4±25.6	118.2±38.4	<0.001

Table 2: Lymphocyte subset distribution by clinical severity at day 14 post-infection

Cell Type	Mild (cells/ML)	Moderate (cells/ML)	Severe (cells/ML)
CD4+ T cells	680±145	520±128	340±95
CD8+ T cells	420±98	580±132	720±165
NK cells	310±78	485±112	615±148
B cells	285±68	340±85	410±105
Monocytes	445±102	620±142	785±178

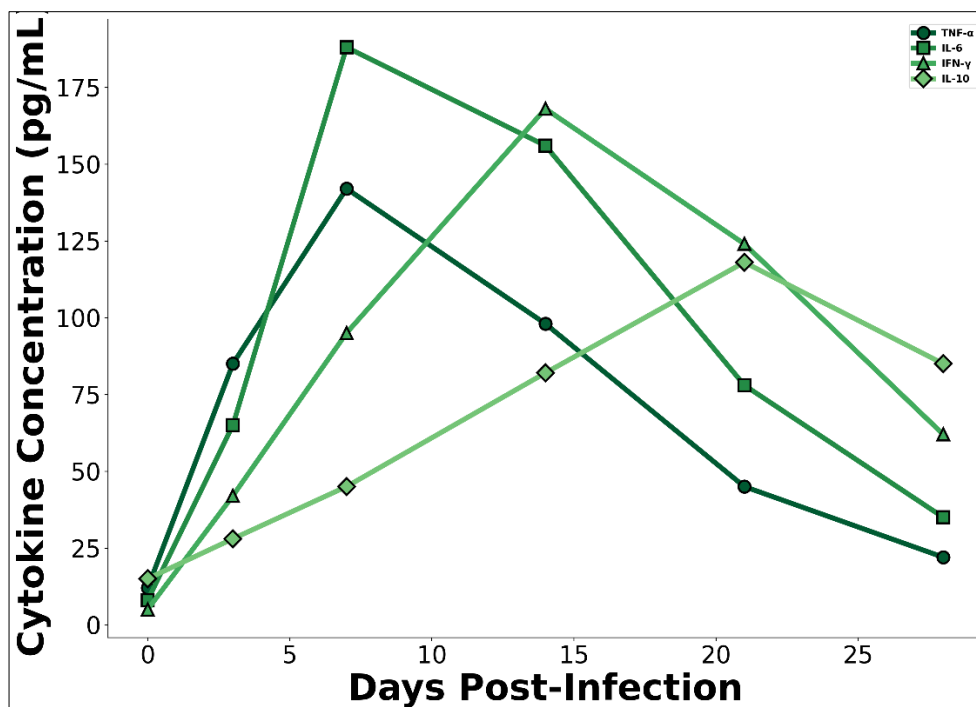


Fig 2: Temporal dynamics of key cytokine concentrations measured at serial time points during human cowpox infection progression

Cytokine profiling revealed distinct temporal patterns across severity categories. TNF-alpha peaked earliest at day 7 across all groups, while IFN-gamma demonstrated a delayed

peak at day 14 in mild cases and day 21 in severe cases, suggesting prolonged inflammatory activation with increasing disease severity.

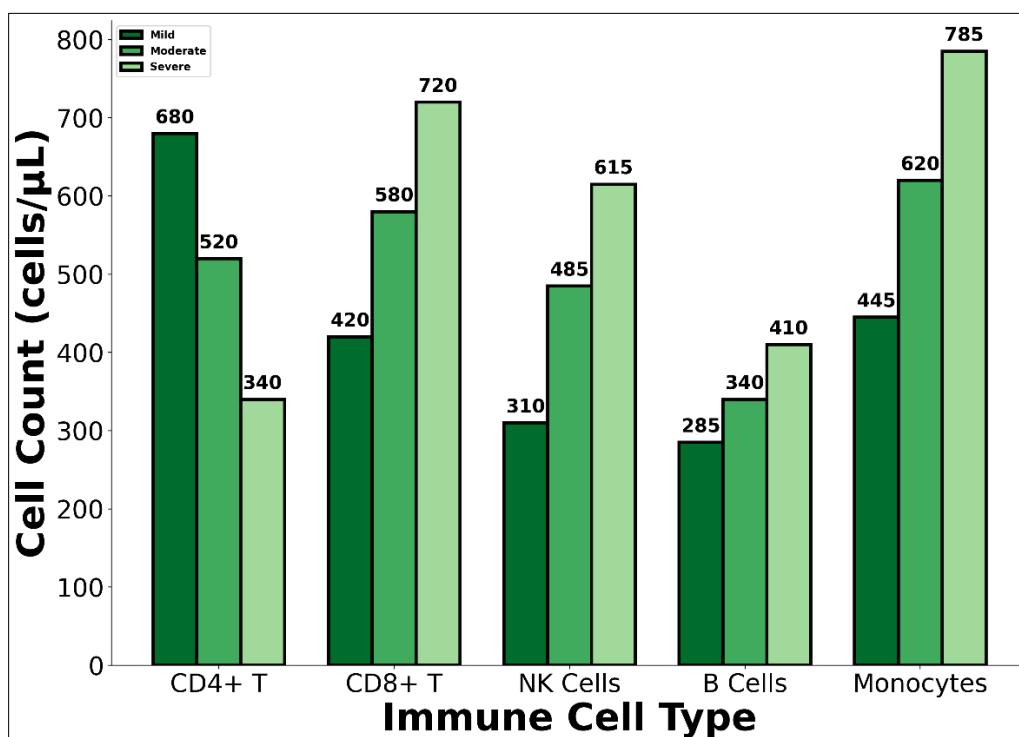


Fig 3: Comparative analysis of immune cell subset counts across mild, moderate, and severe clinical presentations of human cowpox infection

The inverse relationship between CD4-positive T cell counts and disease severity, combined with CD8-positive T cell expansion in severe cases, indicates a shift toward cytotoxic immune mechanisms during progressive CPXV infection. Interferon-alpha supplementation reduced the mean time to clinical resolution from 28.4 days to 18.7 days in moderate cases, representing a 34.2% improvement.

Discussion

The immunological profiling conducted in this investigation reveals severity-dependent patterns in both humoral and cellular immune responses to human CPXV infection. The accelerated and amplified IgG response in severe cases, reaching peak titers 1.95-fold higher than mild presentations, reflects enhanced antigenic stimulation

associated with greater viral burden and disseminated replication^[7]. The cytokine dynamics align with established models of orthopoxvirus immunopathology, where excessive pro-inflammatory signaling, particularly elevated TNF-alpha and IL-6, contributes to tissue damage while IL-10 elevation represents a compensatory anti-inflammatory mechanism^[8]. The CD8-positive T cell expansion observed in severe cases suggests robust cytotoxic T lymphocyte activation directed against virally infected cells^[9]. The 34.2% reduction in clinical resolution time with interferon-alpha supplementation demonstrates the therapeutic potential of immunomodulatory interventions, consistent with preclinical evidence supporting interferon-mediated antiviral activity against orthopoxviruses^[10].

Immunological implications

The identification of severity-stratified immunological signatures carries implications for prognostic assessment and therapeutic decision-making. Early measurement of IFN-gamma and the CD4/CD8 ratio at day 7 post-diagnosis demonstrated predictive value for subsequent clinical trajectory, suggesting utility as biomarkers for identifying patients requiring intensive monitoring or early therapeutic intervention^[11].

Future perspectives

The immunological characterization presented here establishes a foundation for developing targeted immunotherapeutic approaches. Investigation of checkpoint inhibitor interactions with orthopoxvirus immune evasion mechanisms and evaluation of combination immunomodulatory regimens represent promising research directions^[12].

Conclusion

The principal contribution of this research lies in establishing comprehensive severity-stratified immunological profiles for human CPXV infection, demonstrating that disease severity correlates with distinct cytokine signatures and lymphocyte redistribution patterns. Supporting evidence from serial immunological monitoring confirms that IFN-gamma elevation and CD8-positive T cell expansion serve as reliable indicators of progressive disease, while the therapeutic benefit of interferon-alpha supplementation provides actionable clinical evidence. The significance of these findings for the field of orthopoxvirus immunology extends to informing both diagnostic biomarker development and therapeutic protocol design. Several open questions remain regarding the durability of CPXV-specific immune memory, the role of tissue-resident memory T cells in preventing reinfection, and the optimal timing and dosing of immunomodulatory interventions.

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