CASE REPORT

Influenza B-induced Lymphopaenia: A Brief Mechanistic Review.

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Abstract

Influenza is a common respiratory viral infection which is prevalent world-wide. Significant human infections are generally caused by the influenza A and B virus. Influenza often causes an abrupt onset-illness with high fever, constitutional and respiratory symptoms commonly known as 'flu'. Reactive lymphocytosis is the hallmark of most viral infections. However, influenza is commonly observed to present with an early transient lymphopaenia which may be profound.

We present a case of a 20-year-old student diagnosed with symptomatic influenza B who presented with a lymphocyte count of 0.6 x 10^9/L within 24 hours of symptom onset. This article aims to review the current understanding into the mechanisms underlying lymphopaenia development in influenza. Evidence from experimental and human studies are collated and discussed.

Keywords: *cytotoxic T-cells, Fas/Fas ligand, lymphocyte to monocyte ratio, lymphocyte trafficking, mechanisms of lymphopaenia, tumour necrosis factor alpha, viral-induced lymphopaenia.*

Introduction

Influenza is a viral respiratory infection caused by the influenza virus, commonly known as 'flu'. The influenza virus is a negative-sense single stranded RNA virus belonging to the family Orthomyxoviridae. It consists of four genus, denoted as A to D although significant human infections are caused by Influenza A and B. Influenza A affects humans and other animal species and has a very high mutation rate of their haemagglutinin (HA) and neuraminidase (NA) surface antigen. Conversely, Influenza B infection is generally restricted to humans and are genetically more conserved.

The virus spread via droplets or aerosol and mainly infects ciliated columnar epithelial cells lining the human respiratory mucosa. The incubation period following inoculation is approximately 48 hours. The symptom onset is usually abrupt and classically, respiratory symptoms peak around day three before convalescence, which may take over two weeks [1]. The symptoms include constitutional symptoms such as high fever, chills, myalgia and respiratory symptoms (sore throat, dry cough, nasal discharge). In children, gastrointestinal symptoms may be prominent (nausea, vomiting, abdominal pain, diarrhoea). Most infections are uncomplicated and experience uneventful recovery. In a minority of patients, influenza may be complicated. Influenza-associated complications commonly affects high-risk population groups which include pregnant women, children below 5 years, adults over 65 years, immunosuppressed patients and patients with chronic co-morbidities particularly respiratory diseases. Table 1 summarises the influenza-associated complications.

Most viral infections will trigger a lymphoid immune response (clonal expansion of antigen specific T and B-lymphocytes) to eradicate the virus, resulting in a lymphocytosis. Peculiarly, influenza may present with a transient lymphopaenia which can be profound, lasting for several days. Despite this common observation, the mechanism underlying the development of lymphopaenia in this setting is not well known.

Case Presentation

20-year-old university student who was previously fit and well presented with an abrupt onset of fever with rigors associated with myalgia, nausea and vomiting for 24 hours. Several of his flat mates were affected with a similar pyrexial illness over the past week. He denied headaches, dyspnoea, chest discomfort, coryzal symptoms, abdominal pain or diarrhoea. He was not aware of mosquito fogging in his housing area and denied any recent travel abroad or recreational travel to forested waterfall-areas.

Clinical examination revealed a blood pressure of 80/40 mmHg with a pulse of 115/minute, respiratory rate of 34/minute, oxygen saturation of 99% on room air and a temperature of 39.9°C. He was warm to touch with cutaneous flushing. There was a mild non-suppurative pharyngitis without petechiae. There were no tonsillomegaly, cervical lymphadenopathy or facial tenderness present. Auscultation revealed normal breath and heart sounds. Abdominal examination was unremarkable. His neurological examination was grossly normal with absence of meningism. Apart from generalised flushing, there were no cutaneous rashes present.

His basic laboratory investigations revealed a haemoglobin concentration of 14g/dL, leukocyte count 6.4 $x10^9$/L, platelets 252 x 10^9/L, sodium 135 mmol/L, potassium 3.7 mmol/L, urea 5.0 mmol/L and creatinine of 135 µmol/L. The leukocyte differential counts revealed a significant lymphopaenia (absolute lymphocyte count of 0.6 x 10^9/L). The other differential counts were satisfactory (neutrophils: 4.9 x 10^9/L, monocyte 0.8 x 10^9/L and eosinophils 0.03 x 10^9/L) with a lymphocyte to monocyte ratio of 0.75. His dengue NS1 antigen, IgM and IgG were negative. Peripheral blood film to assess for parasitaemia and atypical lymphocytes were not performed. His nasopharyngeal swabs were

positive for Influenza B but negative for SARS-CoV-2 (COVID-19) and influenza A virus (via rapid antigen testing).

A diagnosis of Influenza B was yielded based on the clinical assessment and positive swab results. He was commenced on supportive management with intravenous fluids, paracetamol and metoclopramide. He was given a total of 1.5 litres of normal saline with improvement of his blood pressure to 110/70 and reduction of his pulse to 76/minute. Once his nausea subsided, he was commenced on oseltamivir 75mg twice daily. He clinically improved rapidly over the course of 24 hours with normalisation his physiological parameters and symptom abatement. He was discharged to complete a five-day course of oseltamivir.

Discussion

Transient lymphopaenia is a common feature in influenza A infection (both seasonal and H1N1 pandemic variants) [5] [6]. Greek studies by Merekoulias *et al* identified 85% of confirmed symptomatic Influenza A positive patients demonstrating a lymphocyte to monocyte ratio of less than 2.0 and proposed for the utility of this ratio as a screening tool for Influenza A infection [7]. There were no adult studies identified for this phenomenon in Influenza B infection but paediatric studies by Zhu R *et al* demonstrates a similar phenomenon also occurring among Influenza B positive patients [8].

Despite being a common observation, the mechanism of lymphopaenia development in this infection has not been fully established. In the 1990's, experimental studies on cultured lymphocytes revealed apoptosis to be the mechanism of cell death in influenza A and B exposed lymphocytes [9]. Further experimental studies undertaken by Nichols JE *et al* demonstrated that lymphocyte apoptosis of Influenza exposed cells was mediated via the Fas/Fas ligand interaction [10]. Interestingly, it was revealed that cytotoxic T-cells ($CD8^+$) were the lymphocyte subset that was predominantly affected by apoptosis as these T-cell subset upregulate their surface Fas (CD95) expression upon exposure to the influenza virus. Additionally, upon viral exposure, Fas ligand expression was predominantly increased in cells of the monocyte-macrophage lineage ($CD14^+$ cells). Depletion of $CD14^+$ cells from the culture, reduces lymphocyte apoptosis. This indicates $CD8^+/CD14^+$ cell interaction as the dominant factor in mediating lymphopaenia in influenza A infection [10]. The mechanisms highlighted above could account for the reduced lymphocyte to monocyte ratio observed in Influenza infection. Fascinatingly, the mechanism for influenza exposed lymphocyte apoptosis described by Nichols JE *et al* is identical to the mechanism of uninfected $CD4^+$ T lymphocyte apoptosis in human immunodeficiency virus (HIV) infection described by Badley AD *et al* in the mid- 1990's [10]. In fact, the studies conducted by Badley AD *et al* was the first to demonstrate Fas ligand expression on monocyte derived macrophages [11]. Prior to this study, Fas ligand was thought to be expressed only in lymphoid cells.

Despite the mechanistic evidence of lymphocyte apoptosis elucidated above, the regulator mediating lymphocytic Fas and macrophage Fas ligand expression is still currently unknown. Possible candidates include viral protein antigen, T-cell activation and cytokines released following infection [11].

A more recent experimental study has identified 'influenza A neuraminidase antigen' expressed by infected $CD14^+$ cells to be the regulator mediating $CD3^+$ lymphocyte (unspecified T-cells) Fas expression, ultimately leading to apoptosis [12]. In this study, $CD14^+$ cells were directly infected ex vivo and subsequently expressed viral neuraminidase antigen on its cell surface. Interaction between these infected $CD14^+$ cells and $CD3^+$ lymphocytes subsequently induced apoptosis in the latter. It is unknown if this is truly the mechanism driving lymphocyte apoptosis. In reality, $CD14^+$ cells are not the cells directly infected by influenza but are recruited to the inoculated area by cytokines released by infected

respiratory epithelial cells. Furthermore, these $CD14^+$ cells would have differentiated into macrophages before exerting their effector function. If infected cells are subsequently phagocytosed by these macrophages, the viral antigen would be presented in conjunction with human MHC molecules and not bare surface viral antigen hence casting some doubts into this experimentally proposed mechanism.

In humans, circulating mature T-lymphocytes employ the Fas/Fas ligand apoptosis pathway to regulate their total number in peripheral blood. After antigenic activation, antigen-specific T-lymphocyte undergo clonal proliferation, mediated by interleukin-2. After several cycles of proliferation, the daughter clones will start expressing Fas on its cell surface, making them susceptible to apoptosis upon re-encountering its antigen [13]. This mechanism of action is partly responsible for T-cell exhaustion that is seen with overwhelming viral infections such as chronic hepatitis B and HIV. However, this mechanism is unlikely to explain the lymphopaenia occurring in influenza as infection resolves naturally within a week in the vast majority of cases.

It is now known that specific pathogen generates a unique response by the infected cell and surrounding milieu which triggers the adaptive immune system ($CD4^+$; T-helper cells in this case) to differentiate and polarise into T-helper (T_h) subset function. It's known that cytokines released by these polarised Th cells can regulate not just function of other immune cells but can also modulate proliferation or regression (apoptosis) of these cells. The typical cytokine profile in acute influenza is an elevated interleukin-6 and tumour necrosis factor alpha (TNF-α) which is balanced by an elevated interleukin-10 [14]. This cytokine profile indicates a dominant T_h1 subset immune response. TNF-α may be a potential candidate as an apoptotic regulator. TNF-α together with interferon-gamma has been shown to synergistically induce Fas expression in murine embryonic fibroblasts [15]. However, it is currently unknown if a similar pathway is present in human haematopoietic cells. Further *ex vivo* studies to assess human T-lymphocytic Fas expression in response to various cytokine profiles are required to clarify the contribution of this mechanism.

Other possible mechanisms contributing to lymphopaenia in Influenza include:

1. Physiological lymphocyte trafficking to the affected area (inoculated tissue and regional lymph nodes). There is good evidence that cytotoxic T-lymphocyte activity is required for optimal clearance of the influenza virus (from human and experimental murine studies) [16] [17]. Human studies suggest that influenza specific $CD8^+$ T-lymphocytes takes approximately 3 days to be activated [16]. These lymphocytes will be trafficked to the inoculated tissue in the early part of the infection to eliminate infected epithelial cells. Influenza specific B-lymphocytes are also trafficked to the affected area [18] (to subsequently provide a delayed antibody-mediated clearance and protection). This mechanism may explain the rapid emergence of symptoms and the transient early lymphopaenia observed in influenza infection.

2. Potential reporting error of the automated haematology analyser: It is recognised that activated lymphocytes (previously known as atypical lymphocytes or Downey cells) expand their cytoplasm to a typical size of 15 to 30 µm [19] compared to a resting lymphocyte size of 6 to 8 µm. A typical monocyte measure 16 to 20 µm. Theoretically, automated haematology analysers using the electrical impedance technique for volumetric cell analysis may mistakenly identify an activated lymphocyte as a monocyte. This may erroneously lead to a reduced lymphocyte to monocyte ratio.

However, this is an unlikely situation for 2 main reasons:

a. Most modern laboratories nowadays utilise 'volume conductivity scatter' technology to assess differential leukocyte counts which would clearly distinguish activated lymphocytes from monocytes on the scattergram.
b. Presence of peripheral blood activated lymphocyte (PBAL) is not a feature of influenza infection. An American group has investigated the prevalence of PBAL among patient's presenting with 'influenza -like-illness' (ILI). This study revealed that none of the influenza positive patients had PBAL [20]. In fact, PBAL is a negative predictor for influenza among patients presenting with ILI. Furthermore, PBAL are strongly associated with infectious mononucleosis-like infections which generally presents with a lymphocytosis.

Lymphopaenia is a recognised feature for certain viral infections other than influenza. Table 2 summarises the currently known mechanisms for viral-induced lymphopaenia.

Conclusion

Early transient lymphopaenia is a recognised feature of influenza infection. The mechanism for lymphopaenia in this setting most likely reflects a combination of peripheral tissue lymphocyte trafficking and activation-induced apoptosis via the Fas/Fas ligand interaction.

Table 1. Summary of influenza-associated complications. Adapted from Seminar Influenza [2]

	Complications	**Notes**
Upper respiratory	Otitis media, parotitis, sinusitis, laryngotracheobronchitis.	Commoner in children.
Lower respiratory	Bronchiolitis, bronchitis, pneumonia, acute respiratory distress syndrome, ventilatory failure.	Bronchiolitis is commoner in children. Penumonia may be primary viral or secondary bacterial (including *Staphylococcus aureus*).
Cardiac	Myocardial infarction, heart failure, myocarditis, pericarditis.	Influenza myocarditis is rare. Fulminant myocarditis has a high mortality (24 to 27%) [3].
Gastrointestinal	Hepatitis, pancreatitis, abdominal pain.	Abdominal pain may be due to visceral inflammation or mesenteric adenitis.
Musculoskeletal	Myositis, rhabdomyolysis.	Commoner in children. Myositis (more strongly associated with Influenza B) Rhabdomyolysis (rare; more strongly associated with influenza A) [4].
Renal	Acute kidney injury.	Hypovolaemia or acute tubular necrosis.
Neurological	Seizures, encephalopathy, meningoencephalitis, stroke, transverse myelitis, Guillain-Barré syndrome, Reye's syndrome.	Reye's syndrome is recognised in children receiving salicylates for viral illnesses (stronger association with influenza B). Presenting as encephalopathy with deranged liver function.

Table 2. Summary of known mechanisms of viral-induced lymphopaenia.

Mechanism	Viral infections	Cell types affected	Notes
Direct cytopathy (viral-induced apoptosis)	HIV	$CD4^+$ T cells	Reduced by HAART
	Coronaviruses (MERS-CoV, SARS-CoV-1, SARS-CoV-2)[21], [22]	$CD4^+ > CD8^+$ T cells	
Activation-induced cell death (Fas/FasL interaction)	HIV	$CD4^+$ T cells	Bystander cell affected (reduced by HAART)
	Influenza A	$CD8^+$ T cells	Bystander cell affected
Pyroptosis	SARS-CoV-2[23]	$CD4^+ > CD8^+$ T-cells	Ameliorated by tocilizumab
Autophagy-induced apoptosis	Ebola virus[24]	$CD4^+$ T cells	Infected cells affected
Cytotoxic T-cell mediated destruction	HIV	$CD4^+$ T cells	Antibodies (IgG) are produced against gp14 and gp120 epitope but are non-neutralising[25]
Co-inhibitory molecule expression (CTLA-4, PD-1)	HIV	$CD4^+ > CD8^+$ T-cells	Antigen specific T-cells
	Hepatitis B	$CD4^+ > CD8^+$ T-cells	Antigen specific T-cells
	Hepatitis C	$CD4^+ > CD8^+$ T-cells	Antigen specific T-cells
Lymphocyte trafficking	SARS-CoV-2[26]	$CD4^+$ T cells	
	Influenza A[18]	$CD3^+$ T-cells (unspecified), B-cells ($CD20^+$), NK-cells ($CD56^+$)	

Abbreviations: HIV (human immunodeficiency virus), MERS-CoV (middle-east respiratory syndrome coronavirus), SARS-CoV (severe acute respiratory syndrome coronavirus), HAART (highly active anti-retroviral therapy), IgG (immunoglobulin G), CTLA-4 (cytotoxic T-lymphocyte-associated protein-4), PD-1 (programmed cell death protein-1), NK (natural killer).

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