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In vitro generation of influenza-specific polyfunctional CD4$^+$ T cells suitable for adoptive immunotherapy

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Abstract

Background aims. Influenza viruses cause potentially fatal respiratory infections in stem cell transplant patients. Specific T cells provide long-lived host adaptive immunity to influenza viruses, and the potential for generating such cells for clinical use was investigated. *Methods*. The inactivated influenza vaccine (Fluvax) approved for human use was used as the antigen source. Monocyte-derived dendritic cells pulsed with Fluvax were used to stimulate autologous peripheral blood mononuclear cells (PBMC) on days 0 and 7. Cells were expanded with interleukin (IL)-2 from day 7 onwards. Cell numbers and phenotype were assessed on day 21. The presence of influenza virus-specific cells was assessed by cytokine production and proliferative responses following restimulation with influenza antigens. *Results*. Over 21 days of culture, a mean fold increase of 26.3 in cell number was observed ($n = 7$). Cultures were predominantly effector and central memory CD4$^+$ cells, and expressed a phenotype characteristic of activated antigen-specific cells capable of B-cell helper function. Cytotoxic CD4$^+$ and CD8$^+$ cells specific for influenza and a high percentage of CD4$^+$ cells specific for each of three influenza viruses targeted by Fluvax (H1N1, H3N2 and Brisbane viruses) were generated. In addition, T cells expanded when restimulated with antigens derived from influenza viruses. *Conclusions.* We have demonstrated a clinically usable method for producing influenza virus-specific T cells that yield high numbers of highly reactive CD4$^+$ cells suitable for adoptive immunotherapy. We propose that reconstructing host immunity through adoptive transfer of influenza virus-specific T cells will reduce the frequency of influenza-related deaths in the period of severe immune suppression that follows stem cell transplantation.

Key Words: *adoptive immunotherapy, immune reconstitution, influenza virus-specific T cells, stem cell transplantation, T-cell therapy*

Introduction

Influenza viruses, including the pandemic H1N1 2009 virus, are a common cause of potentially fatal infections in recipients of stem cell transplants (1–3). The severe defect in specific immunity to influenza viruses in recipients of allogeneic hematopoietic stem cell transplantation (HSCT) can persist for up to a year post-transplant, as evident by the poor response to the seasonal influenza vaccination, especially within the first 6 months following HSCT (4,5). While full recovery of the immune system can take up to a year, this period can be extended by more prolonged immune suppression for the prophylaxis or treatment of graft-versus-host disease (GvHD). Over the last decade, increased awareness and aggressive infection control precautions have reduced transmission of influenza (6). However, infection outbreaks in bone marrow transplant (BMT) units have occurred up to and including 2010, with mortality greater than 50% in the HSCT setting (7,8). Antiviral resistance is common (7,9), making influenza a serious opportunistic infection in HSCT patients when combined with its high prevalence in the community during influenza season. In HSCT patients identified with H1N1 infection, approximately 40% progress to lower respiratory tract infections, with mortality of almost 100% at 100 days post-infection (10). Apart from direct morbidity and mortality, respiratory viral infections are a risk factor for the development of invasive fungal infections in allogeneic HSCT patients (11).

Cell-mediated and humoral responses both constitute vital functional elements of host adaptive immunity to influenza infections. Anti-influenza immunoglobulin (Ig) levels are indicative of a seroresponse against influenza vaccination or infection, and antibody-based vaccines towards influenza surface glycoproteins hemagglutinin (HA) and

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neuraminidase (NA) can effectively combat seasonal influenza infections. However, rapid antigenic drift within the HA and NA proteins requires the vaccine to be updated annually, and such vaccines are not effective in the event of an influenza pandemic resulting from a novel strain. Therefore, B-cell mediated humoral antibody responses, although essential for the clearance of infection, are short-lived and strain-specific as cross-reactive antibodies are usually not detected in the event of a pandemic (12,13). Increasingly, the role of T cells in mediating anti-influenza immunity is being appreciated and, in the absence of robust cross-reactive antibody responses, specific T cells elicited against conserved viral epitopes may mediate immune protection against influenza. Indeed, specific $CD4^+$ cells generated following exposure to seasonal influenza viruses and vaccines have been shown to exhibit reactivity to antigenic epitopes derived from live H1N1 virus and are rapidly recruited following subsequent infection with a pandemic strain (14). More recently, it has been suggested that alternative vaccine strategies based on conserved T-cell epitopes may offer broad and lasting immunity against variant influenza strains (15). The presence of T cells capable of producing interferon (IFN)-γ in response to influenza antigens correlates with effective immune protection in the event of an influenza pandemic (12–14). T cells generated by exposure to influenza viruses are therefore thought to possess immunological memory and long-lived strain- and subtype-independent cross-reactivity, and assist in the generation of antibody responses to subsequent infections (12,16).

CD4 T_{helper}-cell interaction with B cells occurs in extra- or intrafollicular regions, resulting in induction of short- or long-lived plasma cells, respectively (17,18). Both these responses have been shown for influenza virus infections, and the induction of long-lived humoral immunity is a result of T_{helper} intrafollicular-dependent germinal center reactions (18). It is therefore conceivable that adoptive transfer of T cells specific for influenza viruses will reconstruct protective host immunity to influenza viruses, including the generation of humoral immune response during infection. In a clinical study of patients with multiple myeloma undergoing autologous stem cell transplantation, *in vivo* vaccine-primed T cells harvested pre-transplant and reinfused post-transplant successfully restored post-transplant influenza vaccine serological responses (19). We studied the *in vitro* generation of influenza virus-specific T cells from normal donors, using procedures that would render the cellular product suitable for adoptive transfer into allogeneic stem cell transplant recipients with severe cellular immune suppression.

Table I. Donor HLA type, expansion and phenotype of cultures.

	A	B	DRB1	Fold expansion	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)	CD14 (%)	CD56 (%)	T_{em} (%)	T_{cm} (%)	T_n (%)	T_{reg} (%)
Donor 1	1,29	8,63	4,15	17.9	98.9	3.6	95.1	<1	<1	2.4	64.2	26.5	1.3	1.8
Donor 2	1,3	8,65	03,13	22.9	98.4	98.1	1.2	<1	<1	1.3	75.7	22.6	0.1	5.1
Donor 3	24,68	65	01021,13	30.8	92.1	42.1	24.6	<1	<1	11.1	67.0	30.3	4.0	0.6
Donor 4	3,26	7,55	14,15	15.9	98.4	54.1	42.2	<1	<1	0.9	67.2	17.4	2.1	10.2
Donor 5	2,3	7,47	7,15	54.4	94.2	90.3	9	<1	<1	1.8	44.9	28.5	11.7	20.1
Donor 6	3,68	35,51	01011,0103	24.5	98.8	89.6	9.1	<1	<1	6.1	35.4	38	9.1	2.9
Donor 7	1,3	7,18	3,15	17.8	68.4	77.6	14.6	<1	<1	30.2	44.1	23.6	6.5	4.5
Mean ± SD				26.3 ± 13.4	92.7 ± 11.1	78.1 ± 21.8	14.9 ± 14.3	0.2 ± 0.2	0.1 ± 0.1	7.7 ± 10.6	56.9 ± 15.2	24.6 ± 7.5	4.6 ± 4.5	6.4 ± 6.8