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ORIGINAL ARTICLE

Serological immune responses to influenza vaccine in patients with colorectal cancer

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Abstract
Background The immune responses to influenza vaccination in patients with colorectal cancer on surveillance or active chemotherapy have not been previously reported. We conducted a prospective influenza vaccination study to determine the serological immune response rate in patients with colorectal cancer.
Methods During the 2006–2007 influenza season, patients with colorectal cancer treated at Roswell Park Cancer Institute were offered vaccination with the trivalent influenza vaccine (Fluzone®, 2006–2007). Blood samples for hemagglutination inhibition (HI) assay titers were collected before and 3 months after vaccination. Response to vaccination was determined using an endpoint of ≥1:40 HI titer ratio or a fourfold HI increase at 3 months from vaccination. A response in HI to at least one of the 3 strains was considered an immune response.
Results Eighty-five patients with colorectal cancer participated in the study. The immune response in the overall population was 70.6%. No differences in response were noted between the 58 patients on active chemotherapy and the 27 patients on surveillance [Odds Ratio (OR) = 0.78; $P = 0.8$]. The odds of response did not vary by chemotherapy regimen or by chemotherapy-vaccination timing. HI response in all 3 titers concurrently were low in both the chemotherapy (12.1%) and surveillance groups (11.1%) (OR = 1.10; $P = 1$).
Conclusions Patients with colorectal cancer mount an immune response to influenza vaccination irrespective of their chemotherapy regimen or timing. However, concurrent responses to all three strains in the individual patient with colorectal cancer are uncommon. The investigation of a booster vaccine in this population is warranted.

Keywords Influenza · Colorectal cancer · Titer · Vaccine

Introduction

Influenza occurs in distinct outbreaks of varying extents nearly every year, mainly in the winter season. This pattern reflects the changing nature of the antigenic properties of influenza viruses. The elderly and those with underlying medical illnesses appear to be particularly vulnerable populations for influenza infection. Patients with cancer, whether receiving chemotherapy or not, are considered at higher risk for influenza infection and its complications. Influenza-related death occurred in an estimated 9% of patients with cancer that were hospitalized [1]. It is currently recommended that patients with chronic medical conditions, including patients with cancer, undergo influenza vaccination on a yearly basis.[1]

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[1] www.cdc.gov/flu/weekly, last accessed on 9/22/2009.

Despite the current universal recommendations, significant limitations exist in our understanding of the responses to the influenza vaccine in patients with cancer and the impact of cancer and/or chemotherapy on the patient's ability to respond to the vaccine. Most of the studies have shown conflicting data on the efficacy of vaccination in this population [2–4]. Published studies have been limited by their patient and treatment heterogeneity and by their small sample size, therefore making it difficult to derive clear and universal conclusions [5]. Furthermore, the clinical benefit from immunization is difficult to quantify due to the lack of a consensus on a definitive surrogate of response. Recent studies have used different time endpoints to assess response to vaccination. A review of the literature shows that some studies use a direct comparison of pre- and post-immunization serum antibody concentrations, others compare geometric mean titers, while others consider a fourfold increase in antibody titers as an indication of vaccine efficacy [3, 6–10]. Adding more complexity is the fact that some consider a response in post-immunization HI titers as a response in any single strain while others require a response in all three strains. In reality, these surrogate laboratory endpoints do not necessarily reflect clinical endpoints; prevention of influenza would be the desired effect.

The influenza vaccine is composed annually of antigens from the strains of influenza that are anticipated to dominate in the coming year. The antigen and strain composition in the Northern hemisphere are determined annually in accordance with the World Health Organization. This information is updated weekly during the influenza season and is available through the CDC.[2] The vaccine is inactivated and is regarded as safe in immunocompromized individuals.

Given the limited information on the response to the influenza vaccine in patients with colorectal cancer, we conducted a prospective vaccination study in a single institute to assess the serological response to the trivalent influenza vaccine in this immunocompromized population.

Methods

Patient selection

In the 2006–2007 influenza season, patients with colorectal cancer seen at Roswell Park Cancer Institute oncology outpatient clinic were asked to participate in a study to assess the serological responses to a trivalent influenza vaccine (Fluzone®, 2006–2007). Institutional review board (IRB) approval was obtained, and patients gave written informed consent to blood collection for hemagglutination inhibition (HI) assays before vaccination and 3 months post-vaccination to assess immune response to influenza vaccination. Baseline patient demographics including age, sex, stage, and type of chemotherapy used were collected.

Fluzone vaccine was administered intramuscularly (IM) to all study patients. Fluzone®, Influenza Virus Vaccine, (Zonal Purified, Subvirion) for intramuscular use, is a sterile suspension prepared from influenza viruses propagated in embryonated chicken eggs. Fluzone vaccine is formulated to contain 45 micrograms (μg) hemagglutinin (HA) per 0.5-mL dose, in the recommended ratio of 15 μg HA each, representative of the following three prototype strains: A/New Caledonia/20/99 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004.[3]

Sample collection and HI assay

Serum samples were collected within 1 week before vaccination and 3 months (±2 weeks) post-vaccination in all study participants. Serum samples were collected and frozen immediately at −80°C through the institutional data banking and biorepository (DBBR). All participating patients had signed an IRB-approved DBBR protocol. Upon completion of study accrual, 300 microliters of serum sample aliquots were shipped on dry ice to the Glennan Center for Geriatics and Gerontology (Norfolk, Virginia) for hemagglutination inhibition (HI) assay. The HI assay measures the serum antibody titer to the influenza virus. Serum containing antibodies to the virus are able to inhibit the agglutination of guinea pig red blood cells (GPRBC's). When a standardized concentration of virus is mixed with a certain and constant amount of GPRBC's, agglutination of the GPRBC's will normally occur. Agglutinated cells have a distinct pattern of settling which is quite different from non-agglutinated cells, and recognition of this pattern of settling is used to determine the antibody titers of the influenza virus. The dilution at which non-specific inhibition is noted, and the next higher dilution of receptor destroying enzyme RDE is used for the assay. The antibody titer is reported as the lowest serum dilution that inhibits agglutination.

Measures of response

Response to vaccination was determined using an endpoint of ≥1:40 titer ratio or a fourfold increase in HI titer at 3 months post-vaccination in any of the three strains.

[2] www.cdc.gov/flu/weekly, last accessed on 9/22/2009.

[3] http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm112854.htm, accessed on 9/22/2009.

Table 1 Patients with colorectal cancer characteristics

	Overall population ($n = 85$)	Chemotherapy ($n = 58$)	Non-chemotherapy ($n = 27$)
Patients' demographics			
Gender (male/female)	48/37		
Age in years: median (range)	61 (23–86)		
Stage IV	49 (57%)	45 (78%)	4 (15%)
Stages I – III	36 (43%)	13 (22%)	23 (85%)
Oxaliplatin-based chemotherapy	27 (32%)	27 (47%)	NA
Irinotecan-based	14 (16%)	14 (24%)	NA
Fluoropyrimidine monotherapy	17 (20%)	17 (29%)	NA
Non-chemotherapy	27 (32%)	NA	27 (100%)

NA non-applicable

Statistical analysis

Analysis was completed in the R statistical computing environment. *P* values were obtained using Fisher's exact test, and the odds ratios are the corresponding conditional maximum likelihood estimates.[4]

Results

Patients' demographics

Eighty-five patients with colorectal cancer were enrolled on study between October and December of 2006. Paired sera (pre- and post-vaccination) were available for all enrolled patients. The median age was 61 years (range 23–86 years). Forty-eight (56%) were male patients and 49 (57%) had metastatic disease at the time of vaccination. Patients were stratified into a "chemotherapy" and "non-chemotherapy" group. The "chemotherapy" group consisted of patients with cytotoxic chemotherapy exposure within 1 month prior to and up to 3 months after vaccine administration. Fifty-eight patients (68%) were on chemotherapy at the time of vaccination. Oxaliplatin-based chemotherapy was the most common chemotherapy regimen used. Baseline patient demographics are detailed in Table 1.

Influenza titer results

Eight (9%) out of 85 patients had a titer of more than ≥1:40 in at least one of the three strains prior to their vaccination. All eight patients were included in the analysis and were considered responders as they experienced a fourfold increase in HI in at least one strain. Figure 1 shows the immune response measured at 3 months post-vaccination in the "chemotherapy" (58 patients) and "non-chemotherapy" (27 patients) groups. Immune response was 70.6% for the overall population, 69% for the chemotherapy (CT) group, and 74.1% for the non-chemotherapy group [Odds Ratio (OR) = 0.78 for CT vs. non-CT groups; $P = 0.8$].

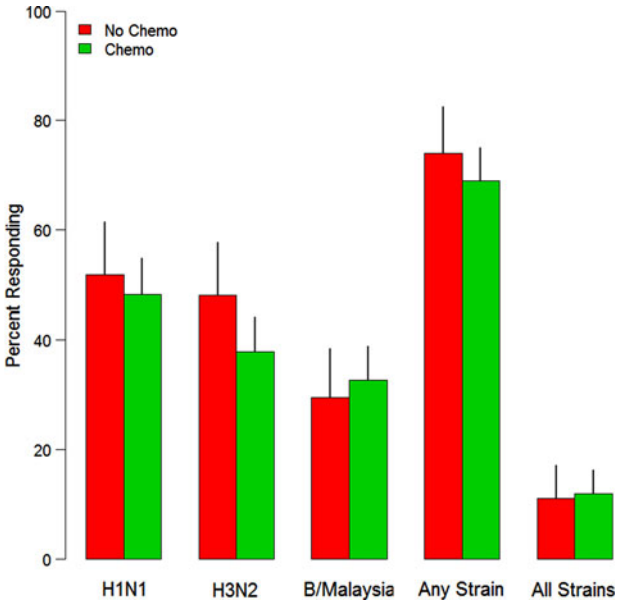

Fig. 1 Immune response to flu vaccine by chemotherapy status. Graph shows percentage of patients expressing immunity (titers ≥ 1:40) to each of the strains, any of the strains, and all of the strains. To at least one of the strains, 70.6% of all patients ($n = 85$) responded : 69% for the CT ($n = 58$) group and 74% for the non-CT ($n = 27$) group [Odds Ratio (OR) = 0.78 for CT vs. non-CT groups; $P = 0.8$]. However, only 11.8% showed response to all three strains: 12.1% in the CT group and 11.1% in the non-CT group (OR = 1.10; $P = 1$). *Tails* indicate SE of the sample percentages

Using a more stringent definition of response that requires titers ≥ 1:40 in all three strains, the response in the chemotherapy and non-chemotherapy groups were 12.1 and 11.1%, respectively (OR = 1.10; $P = 1$).

The chemotherapy group was further divided based on the active chemotherapy given at the time of vaccination:

[4] R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

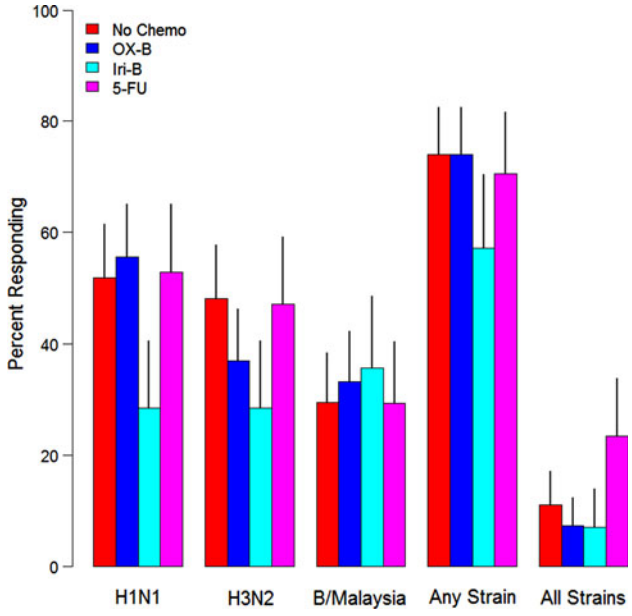

Fig. 2 Immune response to flu vaccine by chemotherapy treatment. Graph shows percentage of patients expressing immunity (titers ≥ 1:40) to each of the strains, any of the strains, and all of the strains. Percentage responding was similar across treatments. Odds ratios relative to non-CT group are 1.0, 0.48, and 0.84, for Ox-B, Iri-B, and 5-FU, respectively. These are not significantly different ($P > 0.05$; see text) from 1.0 or from each other. *Tails* indicate SE of the sample percentages

oxaliplatin-based (27 patients, OX-B), irinotecan-based (14 patients, Iri-B) or fluoropyrimidine-only (17 patients, 5-FU). The odds of response to any one strain did not vary by chemotherapy regimen Ox-B (OR = 1; $P = 1$); Iri-B (OR = 0.48; $P = 0.31$); 5-FU (OR = 0.84; $P = 1$)] when compared to the non-chemotherapy group (Fig. 2). When titer response of ≥1:40 in all three strains was used as a criterion for a vaccine response, no differences in immune response were noted in any of the chemotherapy subgroups [Ox-B (OR = 0.65; $P = 1$); Iri-B (OR = 0.62; $P = 1$); 5-FU (OR = 2.4; $P = 0.4$)]. No differences were noted when we compared the chemotherapy groups based on time of vaccination (vaccination on the day of chemotherapy vs. vaccination more than 1 week after chemotherapy (OR = 0.69; $P = 0.7$) for any strain response or for response in all strains (OR = 1.64; $P = 1$).

Discussion

Influenza vaccination in the general population leads to the development of protective antibody titers in more than seventy percent of vaccinated individuals [11]. Although the value of vaccination in patients with cancer has not been clearly documented, an immune response has been documented, albeit to a varying degree on several clinical trials. Vilar-Compte et al. evaluated the safety and efficacy of influenza vaccine in 146 patients with breast cancer [10]. Overall response to vaccine, defined as a rise in titers to ≥1:40 in all three strains at 4–6 weeks, was 47.2%. In another study by Anderson et al. in 59 patients with lung cancer, 83% developed a titer response 4–6 weeks after vaccination [3]. However, only 12 patients were on active chemotherapy, therefore limiting the assessment of the impact of chemotherapy in this group. Studies in patients with hematological malignancies have reported a considerably lower seroconversion rate [12]. Robertson et al. reported on influenza vaccination in patients with multiple myeloma. In terms of achieving protective levels of antibodies within 6 weeks, the results of vaccination were disappointing with only 14 patients (29%) achieving protection against two or more strains [9]. Vaccination studies in the lymphoma population report conflicting responses to influenza vaccination but a limited response rate has been reported [6, 13]. In an influenza vaccination study involving hematological and non-hematological cancer patients, Nordoy et al. [14] reported an 81 vs. 38% response rate (in at least 2 out of 3 strains) in favor of the solid tumor population. These data suggest an attenuated response in hematological cancers [14].

In our study, we enrolled patients with colorectal cancer treated in a single institute and evaluated the influenza immunological response at 3 months. Influenza vaccination often occurs in September through November while influenza virus exposures occur well through the end of winter. Therefore, ensuring a lasting response to vaccination is necessary in patients at risk. Therefore, for the sake of this study, we selected a 3-month seroconversion rate as the primary endpoint rather than the commonly selected 4–6 weeks endpoint. This may explain the low seroconversion rate for the influenza vaccination when all three strains are considered (12%). Although this percentage is considerably lower than other series of solid tumors, a cross-comparison is not appropriate as other series used a 4–6 week endpoint [10, 14, 15]. Of note, a study by Ramanathan et al. [15] reported on 133 patients with cancer who were evaluated for an immune response to the influenza vaccine at 4 weeks using the same technique and laboratory as our study. The response on Ramanathan et al. [15] study was 41% when the response was defined as an increase in titer of ≥1:40 in all three strains. Our substantially lower response rate could be attributed to our 12-week endpoint. Other possibilities include an inherent difference in the immunogenicity of the influenza vaccine for 2006–2007 in comparison with prior years. Our study still suggests some benefit from vaccination as 70.6% of patients with colorectal cancer had an immunological response to at least one strain at 3 months.

Our findings raise questions regarding the prolonged effectiveness of influenza vaccine and call for serial assessments of influenza titers in at risk populations and the investigation of a second booster influenza vaccine. Indeed, one study has previously shown a significant improvement in seroconversion rates with two influenza vaccine injections in comparison with one in patients with hematological malignancies [16].

Although our study is limited by the lack of a non-cancer population control, we conclude that influenza vaccination in patients with colorectal cancer results in a limited immune response irrespective of the chemotherapy status. Responses to all three strains appear to be uncommon but a response to one strain is encountered in more than two-thirds of the population. Furthermore, the timing of vaccination in relation to chemotherapy administration does not appear to alter the likelihood of response. However, definitive conclusions cannot be made on the relationship between specific chemotherapy regimens or influenza vaccine timing and serological response due to the limited subgroups sample size. At this time, we would recommend influenza vaccination in all patients with colorectal cancer irrespective of chemotherapy status and chemotherapy timing. Larger influenza vaccination studies are warranted in this patient population.

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