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Pre-vaccine plasma levels of soluble inflammatory indices negatively predict responses to HAV, HBV, and tetanus vaccines in HCV and HIV infection

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Abstract

Background—Chronic hepatitis C virus (HCV) and HIV infections are associated with impaired responses to neo-antigens contained in hepatitis A virus (HAV)/hepatitis B virus (HBV) vaccines, yet responsible mechanisms are unclear.

Methods—ACTG 5232 and CFAR0910 were clinical trials where pre-vaccine levels of plasma IP10, IL-6, sCD163 and sCD14 were measured in viremic HCV- (n=15) or HIV-infected participants (n=24) and uninfected controls (n=10). Accelerated dosing HAV/HBV vaccine and tetanus booster were administered and antibody response was measured at 0, 1, 3, 8, and 24 weeks.

Results—Pre-vaccine plasma IP10, IL-6, and sCD14 levels were elevated in both HCV and HIV-infected participants, while sCD163 was elevated in HCV-infected participants. Pre-immunization tetanus antibody levels were lower in HIV-infected than in uninfected participants, while vaccine induced antibody responses were intact in HCV and HIV-infected participants. After HAV/HBV

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vaccination, HCV and HIV-infected participants had lower and less durable HAV and HBV antibody responses than uninfected controls.

Among HCV-infected participants, pre-vaccine plasma IP10, IL-6, sCD14, and sCD163 levels inversely correlated with HAV, HBV and tetanus antibody responses after vaccine. Low HAV/HBV vaccine responses in HIV-infected participants prohibited assessment of immune correlates.

Conclusions—During HCV and HIV infection markers of systemic inflammation reflect immune dysfunction as demonstrated by poor response to HAV/HBV neo-antigen vaccine.

Keywords

HIV; hepatitis C; cellular immunity; T cell; inflammation

Introduction

HCV-infected persons co-infected with HAV or HBV have more severe hepatic damage and increased risk for hepatocellular carcinoma [1], while HIV disease progression and mortality are, in some studies, associated with co-infection with HBV and HCV [2]. Therefore vaccination against HAV and HBV is standard of care for these patient populations. Chronic HCV and HIV infections are associated with impaired responses to many vaccines [3–6] including HAV/HBV vaccines [3–5, 7, 8]. In the case of HCV infection, these impaired responses are not related to cirrhosis or serum HCV RNA levels, suggesting that mechanisms of immune dysfunction unrelated to liver synthetic function or portal hypertension may be involved [9]. During HIV infection, vaccine responses improve with antiretroviral therapy (ART) but remain impaired compared to the responses seen among uninfected controls [8, 10]. During HIV infection, poor vaccine responses are associated with low CD4 T cell counts, low nadir CD4 T cell counts, low resting memory B cells and higher plasma HIV levels [4, 10–12]. Elevated proportions of exhausted T cells have been implicated in reduced responses to vaccine in HCV infection [7].

Elevated plasma levels of inflammatory mediators like IL-6 and soluble CD14 (sCD14) are associated with morbidity and mortality in both HCV and HIV infection [13–19]. Whether these factors relate to vaccine response is unclear. We examined the pre-vaccination levels of soluble inflammatory indices that have been associated with morbidity or mortality in HIV or HCV infection and determined their association with neo-antigen HAV/HBV and recall antigen tetanus booster vaccine antibody responses in untreated HCV, untreated HIV and uninfected control participants in the context of an exploratory clinical trial (AIDS Clinical Trials Group A5332 and the companion clinical trial CFAR 0910). Soluble inflammatory indices that we found elevated in either patient group and were associated with vaccine antibody responses are reported on here. We found that pre-vaccination plasma levels of IL-6, sCD14, sCD163, and IP10 were inversely associated with antibody responses to HAV/HBV vaccines and tetanus booster in HCV and HIV-infected participants.

Methods

Study Participants

AIDS Clinical Trials Group (ACTG) A5232 “Optimizing Vaccine Responsiveness in HIV-1 and HCV Infections by Identifying Determinants of Responsiveness: A Pilot Study” and Case CFAR CFAR0910 “Optimizing Vaccine Responsiveness in HIV-1 and HCV Infections by Identifying Determinants of Responsiveness: A Pilot Study” trials were approved by the institutional review boards at University Hospitals/Case Medical Center, Cleveland VA Medical Center, MetroHealth Medical Center, University of Cincinnati, and ACTG Sites. All participants provided written informed consent in accordance with the Declaration of Helsinki. This was a pilot study specifically designed to identify determinants of vaccine responsiveness to neo-antigen in the form of hepatitis A-hepatitis B immunization (Twinrix[®]) and memory recall antigen in the form of diphtheria/tetanus toxoid immunization (Decavac[™]). Data from the two protocols were combined to examine untreated HCV-infected (n=15), untreated HIV-infected (n=24), and uninfected (n=10) participants. Diphtheria/tetanus toxoid vaccine was administered intramuscular (IM) in the deltoid on day 0; and HAV/HBV (Twinrix[®]) vaccine was administered IM (contralateral deltoid) on days 0, 7, and 21, with booster at 1 year. Participants were age 18–65. They were excluded if they were positive for hepatitis B surface antibody or antigen, hepatitis A antibody, had a known history of HAV or HBV vaccine, were taking systemic antineoplastic treatment, or had therapeutic radiation within 24 weeks of study entry. Chronic HCV-infected participants had detectable HCV RNA in plasma and were without prior anti-HCV therapy. Chronic HIV-infected participants had no contemporary standard of care indication for antiretroviral therapy (ART), or were off ART for at least 3 months and had CD4+ T cell counts >300 cells/mm³.

Vaccine induced antibody levels

Stored serum samples were batch tested for anti-tetanus, -hepatitis A, and -hepatitis B antibody levels at days 0, 7, 21, and weeks 6, 8, 12, and 24 for participants in A5232, and at days 0, 7, 21 and weeks 8 and 24 for participants enrolled in CFAR0910. Anti-Hepatitis B surface (anti-HBs) antibody levels were quantified by ELISA (ADVIA Centaur Anti-HBs2 assay, Siemens Diagnostics, Malvern, PA) at the Cleveland VA Medical Center. Antibody against Hepatitis A antigen was measured by a competitive ELISA using the anti-HAV enzyme immunoassay kit, ETI-AB-HAVK PLUS (DiaSorin, Italy). Tetanus antibodies (IgG) titers were quantified by ELISA (Alpha Diagnostic, Intl, San Antonio, TX).

Soluble inflammatory indices

Plasma IL-6 was measured by high sensitivity ELISA (Quantikine HS, R&D Systems, Minneapolis, MN), and soluble CD14 (sCD14), soluble CD163 (sCD163), and IP-10 were measured by ELISA (Quantikine, R&D Systems, Minneapolis, MN).

Statistical analysis

Continuous variables were compared between 2 groups using the Mann-Whitney U test, and among multiple groups using a Kruskal-Wallis test (GraphPad Prism software, version 5.04).

We assessed the associations between continuous variables by Spearman's rank correlation coefficient, and proportions of groups with vaccine response were compared using Pearson Chi-Square analysis or a Fisher Exact Score (SPSS version 22). Paired week 0 and week 24 tetanus responses were analyzed using a Wilcoxon matched-pair sign-rank test. A "p" value <0.05 was considered significant.

Results

Study participant clinical characteristics

Fifteen untreated HCV-infected, 24 untreated HIV-infected, and 10 healthy control participants were enrolled. Characteristics of the participants are shown in Table 1. HCV-infected participants were older than HIV-infected participants ($p=0.004$) and controls ($p=0.04$). Although all HIV-infected participants had CD4 counts >300 cells/mm³, CD4 T cell counts in this group were less than among uninfected donors and HCV-infected participants ($p=0.023$ and $p=0.0007$, respectively), while HCV-infected participants had higher CD4 T cell counts than among uninfected donors ($p=0.022$). The HIV-infected group had 4 Hispanic participants whose antibody responses to the HBV antigen were particularly low (no response detected).

Antibody responses to HAV/HBV vaccine are impaired in viremic HCV and HIV-infected participants

Vaccine responses to HBV in HCV infection [7, 9] and HIV infection [4, 5] have been previously described as impaired, and our results confirm this (Fig. 1). Participants were examined for antibody responses after receiving accelerated HAV/HBV vaccine given at weeks 0, 1, and 3 and tetanus booster given at week zero. Participants with HAV antibody levels >20 mIU/mL or HBV antibody levels >10 mIU/mL by week 12 or 24 were considered responders. The proportion of HCV-infected participants that responded to the HAV (78%) and HBV component of the HAV/HBV vaccine (73%) by week 24, tended to be lower than the proportion of uninfected participant responders to HAV (100%) and HBV (88%) (Fig. 1). The proportion of HIV-infected participants responding to HAV (88%) and HBV (38%) vaccine by week 24 also tended to be lower than uninfected participants (HAV 100% and HBV 88%) (Fig. 1).

We next evaluated the antibody levels specific to the vaccines. HAV antibody titers were significantly lower in HIV-infected participants at weeks 1, 8, and 24 compared to those in uninfected controls ($p<0.05$, <0.05 , and <0.0001 , Fig. 1A); and change in HAV antibody titers after vaccination were significantly lower at weeks 8 and 24 (supplemental Fig. 1A). At week 24 HAV antibody titers (and change from baseline) were also significantly lower in HCV-infected participants compared to those in uninfected controls ($p<0.05$, Fig. 1A, supplemental Fig. 1A). HBV antibody titers were significantly lower in HIV-infected participants at week 1 compared to those in HCV-infected participants and uninfected controls ($p<0.05$). HBV antibody titers in HIV-infected participants remained lower at weeks 3 and 8 compared to those in HCV-infected participants ($p<0.05$), and at week 24 HBV antibody titers in HIV-infected participants remained significantly lower than those in uninfected controls ($p<0.05$, Fig. 1B). The change in antibody titer from baseline was

significantly lower in HIV-infected participants at weeks 8 and 24 compared to those in uninfected participants (supplemental Fig. 1B) and were significantly lower than HCV-infected participants at weeks 3 and 8 (supplemental Fig. 1B).

Baseline anti-tetanus antibody levels were lower in HIV-infected participants (median 375 U/mL) than among uninfected participants (median 616 U/mL) ($p = 0.004$) (Fig. 1C and D). HCV and HIV-infected participants had a significant increase in tetanus antibody titer in response to vaccine, comparing week 0 and week 24 ($p = 0.004$ and $p = 0.039$ respectively (Fig. 1D). Antibody response to tetanus vaccine was intact in the HCV and HIV-infected groups, but the magnitude of the baseline and week 24 anti-tetanus antibody titers was lower in HIV-infected participants (median baseline 375 U/mL; week 24 465.8 U/mL) than those in uninfected participants (median baseline 616 U/mL; week 24 736.6 U/mL). The baseline and week 24 anti-tetanus antibody titers in the HCV-infected participants tended to be lower (median baseline 491.5; week 24 598.8) than those in uninfected participants but were not significantly lower. The change in tetanus antibody responses from baseline were greater in HCV-infected participants than were changes in response of the uninfected participants (supplemental Fig. 1C) and at week 3 the change in tetanus antibody responses was greater in HIV-infected participants than were changes in uninfected participant responses (supplemental Fig. 1C).

If only maximal antibody responses were considered for each subject, regardless of time, HAV antibody responses were lower in the HCV and HIV-infected participants compared to healthy controls; HBV antibody responses were lower in the HIV-infected participants compared to the healthy controls and the HCV-infected participants; and there was no significant difference in maximal antibody responses to tetanus between the groups (supplemental figure 2).

In the HIV-infected participants, there was no association between CD4 T cell count and vaccine antibody titer/response; nor was there an association between CD4 T cell count and age or inflammation indices. However, in HIV-infected participants, age was inversely correlated with HAV antibody titers at week 24 ($r = -0.480$ $p = 0.047$) and inversely with HBV antibody titers at week 8 and week 24 ($r = -0.378$ $p = 0.002$; $r = -0.528$ $p = 0.004$).

Pre-vaccine plasma levels of inflammatory mediators are elevated in HCV and HIV-infected participants and are associated with lower vaccine response

Systemic inflammation persists [20] and is associated with disease progression during treated HIV infection [16, 17, 19, 21] and untreated HCV infection [22, 23]. In particular, IL-6, sCD14, sCD163, and IP10 levels are elevated in the plasma of individuals with chronic HCV and HIV infection [14, 15, 24–29]. Consistent with prior literature [15, 18, 20, 24], pre-vaccine plasma levels of IL-6 were elevated in HIV and HCV-infected participants compared to levels in uninfected donors (Fig. 2A). Additionally, pre-vaccine plasma IL-6 negatively correlated with HBV antibody levels at weeks 3 and 8 in HCV-infected participants (Fig. 2B). Similarly, pre-vaccine plasma levels of sCD14 were elevated in both HIV and HCV-infected participants, consistent with prior literature [14, 15, 18, 24], and were negatively associated with HAV antibody levels at week 8 after vaccination in HCV-infected participants (Fig. 2C, D). Pre-vaccine plasma levels of sCD14 were also negatively

associated with tetanus antibody titers in HCV-infected participants (Fig. 2E). Pre-vaccine plasma levels of sCD163 were elevated in HCV-infected participants compared to levels in uninfected donors and HIV-infected participants (Fig. 2F), and this was inversely correlated with HBV and HAV antibody levels in HCV-infected participants (Fig. 2G, H). IP10/CXCL10 is an inflammatory chemokine produced by monocytes and endothelial cells in response to type I (IFN α , IFN β) and type II (IFN γ) IFNs [30, 31]. Pre-vaccine plasma levels of IP10 were elevated in both HIV and HCV-infected participants compared to levels in uninfected donors (both $p = <0.001$) (Fig. 2I), and were inversely correlated with tetanus antibody titers in both HIV (week 1) and HCV-infected participants (Fig. 2J, K).

Despite elevated plasma levels of IP10, IL-6, and sCD14 in the HIV-infected participants, levels did not correlate with HAV or HBV antibody responses. Only 4 of 24 HIV-infected participants responded to the vaccine, therefore there were not enough antibody values to see any correlation with plasma levels of IP10, IL-6, or sCD14. Associations between plasma levels of inflammatory indices and vaccine antibody responses were not examined in the uninfected participants due to low sample size ($n=10$).

Discussion

HCV and HIV infection are characterized by chronic systemic immune activation that is associated with increased morbidity and mortality [13, 14, 32, 33]. Those with HCV and HIV infection show reduced responses to vaccination, but the mechanistic underpinnings and the connection with immune activation has been unclear. We demonstrate here that elevated pre-vaccination plasma levels of soluble markers of immune activation (IL-6, sCD14, IP10, sCD163) are associated with lower responses to vaccination with HAV/HBV and tetanus during HCV infection. During HIV infection, elevated levels of plasma IP10 were associated with lower response to tetanus booster.

Plasma levels of IL-6 and sCD14 predict mortality and morbidity even in treated HIV infection [16] [15, 17, 19], and disease progression in HCV infection [13]. We confirm here, elevated levels of IL-6 and sCD14 in HCV and HIV infection and found that plasma levels of IL-6 were inversely associated with HBV antibody responses to HAV/HBV vaccine in HCV-infected participants. CD14 is a monocyte differentiation antigen and a receptor for bacterial LPS. Soluble CD14 is generated upon activation of monocytes [34] and Kupffer cells [35]. We found that pre-vaccination plasma levels of sCD14 were inversely associated with antibody responses to HAV after HAV/HBV vaccination and with antibody responses to the tetanus booster in HCV-infected participants. Activation of monocytic Kupffer cells by HCV may cause the release of sCD14 and IL-6 and contribute to overall immune activation and dysfunction. In HCV infection, where infection is largely compartmentalized to the liver, the Kupffer cell is one likely source of sCD14 [35–37]. In particular, during HCV infection Kupffer cell/monocyte activation appears to be closely related to systemic immune activation and immune dysfunction. Activated monocytes/macrophages are also a source of soluble CD163 [25, 38, 39] and this is associated with fibrosis and liver disease in HCV infection and HCV/HIV coinfection [38, 40] and with coronary plaques in HIV mono-infection [39]. In the HCV-infected participants plasma sCD163 levels were elevated and

associated with lower antibody titers to both HAV and HBV, again potentially linking activation of monocyte/Kupffer cells to immune dysfunction.

IP10/CXCL10 is also produced by monocytes and endothelial cells in response to Type I and Type II IFNs [30, 31]. Plasma levels of IP10 are elevated in HCV infection [27] and are associated with indices of hepatic damage and fibrosis [28, 29, 40, 41]. In HIV infection, plasma levels of IP10 are associated with rapid disease progression early in untreated HIV infection [26]. Here, we found that pre-vaccine plasma IP10 levels were elevated and negatively correlated with antibody responses to tetanus booster vaccination in both HCV and HIV-infected participants. Moreover, in both HIV and HCV-infected participants, plasma IP10 levels were positively correlated with both CD4 and CD8 T cell activation (data not shown).

Mechanistically, how these inflammatory factors (IL-6, IP10, sCD14 and sCD163) might impact vaccine responses is not known. T cells, specifically T follicular helper T cells (Tfh) in lymph nodes are essential for B cell class switching, affinity maturation, and the generation of memory B cells [42]. A study examining influenza vaccine in elderly adults found that age-related changes in circulating Tfh cells were associated with reduced responses to influenza vaccines [43]. Previously, we found reduced numbers of naïve CD4 T cells during HCV infection [44]. A hallmark of HIV infection is reduced numbers of CD4 T cells, functional and anatomical disruption of the lymph nodes [45], and a disruption of the IL-7 axis [46]. Specifically, we demonstrated that PBMCs from uninfected subjects exposed in vitro to the inflammatory cytokines IL-6 and IL-1 β resulted in the down-regulation of CD127, the alpha chain of the IL-7 receptor [46]. Interleukin-7 is particularly important for the expansion of naïve T cells and central memory T cells, the maintenance of both lymph node and peripheral homeostasis, and the prevention of apoptosis [47]. We also demonstrated that PBMCs pre-exposed to IL-6 and IL-1 β showed reduced responses to IL-7 [46]. Therefore, the negative correlation observed between IL-6 and vaccine response may be linked to IL-6 mediated impairment of the “helper” T cell response.

It is plausible that the elevated inflammatory indices seen in the peripheral blood of viremic HIV and HCV-infected participants are also elevated in the lymph node tissues. High levels of IL-1 β or IL-6 in the lymph nodes may affect the survival and function of Tfh cells in lymph nodes and as a result this may influence the generation of B cell antibody responses and B cell memory to vaccination.

When comparing tetanus recall responses to neo-antigen HAV/HBV responses, there was a marked decrease in vaccine responses to the neo-antigen vaccine, HAV/HBV, in the HCV and HIV-infected participant groups. The more subtle reduction in responsiveness to the recall vaccine, tetanus, was reflected by the inability to maintain the response to the booster. It is likely that the infected participants received their initial vaccine to tetanus as children before becoming infected with HIV or HCV and generated T cell memory to tetanus at that time. However, the baseline responses to tetanus were significantly lower in the HIV-infected participants (Fig. 1C, D); perhaps this reflects the loss of CD4 memory T cells during the acute HIV infection. While most of the HCV or HIV-infected participants responded well to the tetanus booster initially (weeks 1 and 3) responses quickly waned

(weeks 8 and 24) (Fig. 1C). This may reflect the dysregulation of T cell/B cell interactions within the lymph nodes, or the inability of the T cells to support B cell memory generation that arose after infection with HIV or HCV [45].

Prior studies suggest that immune exhaustion and senescence is also associated with poor vaccine responses in the elderly [48]. Similar observations during HCV-infection indicate non-response to HBV vaccine is associated with the exhaustion/senescence marker KLRG-1 on CD4 T cells [49], and Tim-3 on monocytes. Recently, we have demonstrated that in vitro exposure of uninfected PBMCs to inflammatory cytokines like IL-1 β and IL-6 can drive T cell expression of exhaustion/senescent markers PD-1, CD57 [50] and Tim-3 (unpublished). These in vitro data may provide one link between elevated inflammatory mediators like IL-6, T cell exhaustion, and immune impairment.

One limitation of this pilot study was that it was not powered to examine associations in the uninfected participants. Another limitation is an inability to identify immune predictors of antibody responses to HAV/HBV vaccination in HIV-infected participants due to the unexpected, very low vaccine response rates in this group. Certainly, the sample size of this pilot study is small, but the study results suggest that the relationship between immune activation and immune impairment warrant further investigation in larger sample sets.

In conclusion, pre-vaccination levels of select soluble markers of immune activation are negative predictors of subsequent vaccine response in the setting of chronic HCV infection. Understanding mechanisms underlying immune impairment during chronic viral infection are needed to guide strategies to improve immune health during these morbid infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Hepatitis A and B specific antibody responses to accelerated HAV/HBV immunization and tetanus booster are impaired in viremic HCV-infected and HIV-infected participants
 Serum was tested for hepatitis A surface protein-specific antibodies (A), hepatitis B specific IgG (B), and tetanus specific antibodies (C and D) by ELISA. A–C) UD= uninfected donors, open bars; HCV+= untreated HCV-infected participants checked bars; HIV+= viremic HIV-infected participants off ART, dotted bars. Bars within boxes represent medians, whiskers are 1–99 percentile; groups compared by Mann-Whitney U test. Antibody responses to HAV ≥ 20 mIU/mL were considered positive and antibody responses to HBV ≥ 10 mIU/mL were considered positive. D) Week 0 and week 24 paired anti-tetanus antibody levels in uninfected donors, HCV-infected, and HIV-infected participants. Paired week 0 and week 24 anti-tetanus antibody titers compared by Wilcoxon match-pair sign-rank test. * $p < 0.05$; ** $p < 0.0001$

Figure 2. Pre-vaccine plasma levels of inflammatory mediators are elevated in HCV and HIV-infected participants and are negatively associated with vaccine antibody responses
 Plasma levels of IL-6 (A), soluble CD14 (C), soluble CD163 (F), IP10/CXCL10 (I), were measured from uninfected donors (UD) (n=10), HCV-infected participants (n=15), and viremic HIV-infected participants off ART (n=24) by ELISA. Antibodies against HAV antigen, HBV surface protein, and tetanus antigen were measured by ELISA. Correlations between HAV, HBV, and tetanus antibody titers and soluble factors (IL-6, sCD14, IP10,

sCD163) were examined using Spearman's rank correlation analysis. Weeks showing a significant correlation are in bold. Between group comparisons of pre-vaccine plasma levels of IL-6, sCD14, IP10, and sCD163 were made using Mann-Whitney U test. $p = <0.05$ considered significant

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Table I

*Kruskal-Wallis statistical test showed a significant difference in age ($p=0.01$) and *CD4 T cell count ($p=0.0009$) between groups. HCV-infected participants were significantly older than uninfected donors (Mann-Whitney $p=0.043$) and HIV-infected patients (Mann-Whitney $p=0.004$). All HIV-infected participants had CD4 T cells counts >300 cell/uL, however, HIV-infected participants had lower CD4 T cell counts than uninfected donors and HCV-infected participants ($p=0.023$ and $p=0.0007$, respectively) and HCV-infected participants had CD4 T cell counts significantly higher than uninfected donors ($p=0.022$). HepB Core Ab+= hepatitis B core antibody positive; AST= aspartate aminotransferase; ALT= alanine transaminase; PLT= platelet count

Group	Uninfected donor	HCV	HIV
Number	10	15	24
*Age(range)	38 (22–62)	53 (22–61)	32 (19–64)
Race			
Caucasian	8/9	7/13	12/24
African American		6/13	7/24
Hispanic			5/24
Asian	1/9		
Sex/Male	5 (50%)	10 67%	19 (79%) 13,000 (n=23)
HIV (c/mL)			(247–100,000)
*CD4 count (Range)	769 (550–1362)	1297 (326–1676)	533 (215–2030)
HCV genotype%			
1		80%	
2		6%	
3		13%	
HCV (IU/mL)		4599222 (256304–24977684)	
HepB Core Ab (+)	0/10 (0%)	6/12 (50%)	0/24 (0%)
AST (IU/mL)	23 (13–56)	43 (23–180)	24 (9–64)
ALT (IU/mL)	32 (20–63)	59 (15–282)	22 (15–51)
PLT ($10^3/\text{mm}^3$)	230.5 (184–297)	220 (74–311)	250 (157–373)