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Research paper

Hapten-specific naïve B cells are biomarkers of vaccine efficacy against drugs of abuse ☆

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ABSTRACT

Vaccination against drugs of abuse shows efficacy in animal models, yet few subjects achieve effective serum antibody titers in clinical studies. A barrier to translation is the lack of pre-vaccination screening assays that predict the most effective conjugate vaccines or subjects amenable to vaccination. To address this obstacle, we developed a fluorescent antigen-based enrichment method paired with flow cytometry to characterize hapten-specific B cells. Using this approach, we studied naïve and activated B cells specific for structurally-related model haptens based on derivatization of the morphinan structure at the C6 position on oxycodone or at the C8 position on hydrocodone, and showing different pre-clinical efficacy against the prescription opioid oxycodone. Prior to vaccination, naïve B cells exhibited relatively higher affinity for the more effective C6-derivatized oxycodone-based hapten (6OXY) and the 6OXY-specific naïve B cell population contained a higher number of B cells with greater affinity for free oxycodone. Higher affinity of naïve B cells for hapten or oxycodone reflected greater efficacy of vaccination in blocking oxycodone distribution to brain in mice. Shortly after immunization, activated hapten-specific B cells were detected prior to oxycodone-specific serum antibodies and provided earlier evidence of vaccine failure or success. Analysis of hapten-specific naïve and activated B cells may aid rational vaccine design and provide screening tools to predict vaccine clinical efficacy against drugs of abuse or other small molecules.

1. Introduction

Drug abuse is a worldwide public health threat (UNODC. World Drug Report, 2012). Current drug addiction pharmacotherapies exhibit limitations and side effects. Medications for cocaine and methamphetamine abuse are lacking and those for treatment of nicotine and opioid addiction exhibit suboptimal clinical efficacy (Harmey et al., 2012; Shorter and Kosten, 2011; Stotts et al., 2009; Skolnick and Volkow, 2012). While vaccines are typically utilized to prevent infection, vaccination against drugs of abuse may offer an alternative to pharmacotherapy for treatment of drug dependence (Shen et al., 2012; Montoya, 2012). Active immunization against drugs of abuse is achieved by conjugating the target drug to a larger immunogenic carrier of bacterial, viral or other foreign origin (Shen et al., 2012). Vaccine efficacy is based on the ability of generating drug-specific serum antibodies that retain drugs in serum, preventing distribution to the brain and subsequent rewarding effects.

Abbreviations: KLH, Keyhole limpet hemocyanin; PE, Red-phycoerythrin; AF, Alexa Fluor 647.

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Despite promising animal studies, vaccines against drugs of abuse have not yet met clinical expectations because few subjects achieved effective serum antibody titers (Kinsey et al., 2010; Kosten et al., 2002; Martell et al., 2009; Hatsukami et al., 2005, 2011; Tonstad et al., 2013; Fahim et al., 2013). Evaluation of vaccines against nicotine or cocaine showed that only ~30% of immunized subjects developed clinically effective drug-specific serum IgG antibody concentrations of at least 40 μg/ml necessary to achieve smoking cessation or abstinence from cocaine (Martell et al., 2009; Hatsukami et al., 2011). It is not understood why current immunization strategies only achieve clinically effective antibody levels in a small subset of vaccinated people. A main barrier to translation is the lack of pre-vaccination screening assays that would predict the most effective vaccines or subjects amenable to vaccination. The goal of this study was to develop a pre-immunization B cell-based screening assay and to test if the pre-immunization naïve B cell repertoire correlates with vaccine efficacy against drugs of abuse.

Previously, we developed vaccines against prescription opioids, which are amongst the most commonly abused drugs in the USA (National Survey on Drug Use and Health: National Findings, 2011; Maxwell, 2011). To target both oxycodone and hydrocodone, we generated a series of structurally-related immunogens containing haptens based on derivatization of the C6 and the C8 position on the morphinan structure, and conjugated to the keyhole limpet hemocyanin (KLH) carrier protein through amide bond or thioether linkage (Pravetoni et al., 2013). Despite the closely-related structures, a C6-derivatized oxycodone-based hapten (6OXY) conjugated through a tetraglycine linker to KLH was more effective than immunogens containing C6- and C8-derivatized hydrocodone-based haptens in blocking both oxycodone and hydrocodone distribution to the brain and their behavioral effects in mice and rats (Pravetoni et al., 2013). The 6OXY hapten generated high titers of high affinity serum antibodies that selectively bound both oxycodone and hydrocodone, while the C8-derivatized hydrocodone-based hapten (8HYDROC), containing an identical tetraglycine linker, generated poorly effective antibody responses against oxycodone (Pravetoni et al., 2013). In the current study, we used the 6OXY and 8HYDROC model haptens to test if the pre-immunization naïve B cell repertoire correlates with vaccine efficacy against oxycodone in mice.

After vaccination, antibodies specific for the target drug are produced only after the successful activation and proliferation of naïve B cells (Shen et al., 2012). Prior to vaccination the host immune system contains millions of "naïve" B cells circulating in the bloodstream through the spleen and lymph nodes. Each naïve B cell expresses a transmembrane form of antibody called the B cell receptor (BCR) that is unique to each cell. A small number of naïve B cells express a BCR that is able to bind the drug hapten within the vaccine. Hapten binding to the BCR on naïve B cells is the first step in a complex process of proliferation and differentiation that generates numerous cell types with different roles in protective immunity (Pape et al., 2011; Taylor et al., 2012a). Short-lived plasma cells secrete antibody early in the response and then die. Germinal center (GC) B cells undergo a process to increase BCR affinity for antigen and these cells mature into long-lived antibody-secreting B cells that maintain serum immunoglobulin levels, or memory B cells (Taylor et al., 2012a; McHeyzer-Williams and McHeyzer-Williams, 2005). Memory B cells may also be generated in a GC-independent pathway (Taylor et al., 2012b). In response to additional vaccinations, the memory B cells will proliferate and generate more antibody-secreting B cells, which boost drug-specific serum IgG antibody levels.

There is little information regarding B cells specific for haptens or immunogens used in conjugate vaccines against drugs of abuse. Here, we have adapted a fluorescent antigen-based enrichment approach to study naïve B cells specific for morphinan haptens from candidate vaccines and to understand their relevance to successful vaccination against prescription opioids. This type of analysis has not been possible until recent development of a method to concentrate the rare cells of interest into a sample small enough to be analyzed in its entirety using flow cytometry (Pape et al., 2011). The sensitivity of this method is such that the number and affinity of antigen-specific B cells can be studied in non-vaccinated animals (Taylor et al., 2012c). Analysis of B cells prior to vaccination showed that naïve B cells exhibited relatively higher affinity for the more effective 6OXY hapten and that the 6OXY-specific naïve B cell population contained a higher number of B cells with greater affinity for free oxycodone than the 8HYDROC-specific naïve B cell subset. Shortly after immunization, activated hapten-specific B cells were detected earlier than oxycodone-specific serum antibodies and their analysis showed that 6OXY-KLH elicited higher numbers of activated hapten-specific B cells than 8HYDROC-KLH. These early pre-clinical findings suggest that it may be possible to identify more effective haptens and immunogens through analysis of their interaction with naïve B cells prior to vaccination. This knowledge would accelerate rational vaccine design based on generation of haptens with high affinity for naïve B cell subsets or help to identify subjects that will develop clinically effective serum antibody levels.

2. Material and methods

2.1. Drugs and reagents

All opioids were obtained through the NIDA Drug Supply Program and Sigma (St. Louis, MO). All drug doses and concentrations are expressed as the weight of the free base.

2.2. Hapten synthesis

The C6- and C8-derivatized morphinan haptens containing an identical tetraglycine linker (6OXY and 8HYDROC) were synthesized as previously described (Pravetoni et al., 2012a, 2013). 6OXY was obtained by the condensation of oxycodone with *O*-carboxymethoxylamine hemihydrochloride in refluxing methanol using pyridine as a base. This intermediate was then coupled to tetraglycine tertbutyl ester (Gly_4tBu) using a N,N′-Dicyclohexylcarbodiimide/hydroxybenzotriazole (DCC/HOBt) procedure followed by acid hydrolysis as described previously (Pravetoni et al., 2012a). 8HYDROC was obtained by addition of thioglycolic acid to codeinone allowing modification at the C8 position to generate the corresponding 8-substituted dihydrocodeinone, which consisted of an unresolvable mixture of 8α- and 8β-epimers. This intermediate was then coupled to the tetraglycine linker resulting in a diastereomeric mixture that was used for conjugation to proteins (Pravetoni et al., 2013).