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Increased immunogenicity to P815 cells modified with malondialdehyde and acetaldehyde☆

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Abstract

Aldehyde modified proteins have been associated with the development and/or progression of alcoholic liver disease (ALD). These protein adducts are capable of initiating many immunological responses that are harmful to the normal homeostasis of organism function. Previous studies have shown that malondialdehyde (MDA) and acetaldehyde (AA) synergistically form a unique adduct (MAA) with soluble proteins, which are capable of inducing cytokine release, T-cell proliferation, and antibody production. The purpose of this study was to determine whether MAA adduction can elicit similar responses to cells using a well-defined tumor model. The mouse mastocytoma P815 tumor cell line was modified with MAA (P815-MAA) or left unmodified (P815) and 10^6 irradiated cells were injected into DBA/2 mice once a week for 5 weeks. Serum was collected and tested for antibody responses to P815 cells and the MAA epitope. Immunization of MAA adducted P815 cells into syngeneic DBA/2 mice induced a strong antibody response to the MAA epitope as determined by ELISA on Alb and MAA-Alb (508 μg/ml and 1092 μg/ml, respectively). In addition, antibody to unmodified P815 cells was detected by fluorescent technique. Mice immunized with P815 cells or PBS showed little or no reactivity to the MAA epitope or P815 cells. Studies to assess IL-12

Abbreviations: MDA, malondialdehyde; AA, acetaldehyde; (IL-12), interleukin twelve; Alb, bovine serum albumin; PBS, phosphate buffered saline.

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stimulation showed that peritoneal macrophages from P815 and PBS immunized animals produced modest amounts of IL-12 (20 and 35 pg/ml) when stimulated with Alb or MAA–Alb. However, macrophage from P815-MAA immunized mice responded to soluble MAA adduct (142 pg/ml). Finally, in tumor survival studies the mean survival was 14.25 days in PBS treated mice; 15.75 days with P815 immunized mice and 18.25 days with P815-MAA immunized mice. Therefore, these data strongly suggest that antibody responses are induced by P815 cells modified with MAA adducts. This may be a possible tool to begin looking at how alcohol metabolites potentially modify cells and/or cellular components making them recognizable to the immune system as foreign. It is thought that these studies define a model system that will be useful in assessing antibody and potentially T-cell responses to cells that are modified by MAA.

1. Introduction

A relationship between the immune system and alcoholic liver disease (ALD) has been reported by many investigators [1–5]. For example, there is an influx of immune cells found in liver biopsies from alcoholic liver disease patients [1]. Additionally, it has been shown that substantial abnormalities occur in the humoral and cellular immune responses of patients with liver disease associated with alcohol [6–10]. While a number of different antigens have been involved in these immune responses, no one epitope has been identified as the true mediator of ALD.

Support for immune involvement in ALD is found in the detection of autoantibodies to various antigens found in the serum of chronic ethanol-fed rats or patients with alcohol-induced liver injury. These autoantibodies include; CYP2E1 [11], alcohol dehydrogenase (ADH) [12] hydroxyl ethyl free radicals [13], acetaldehyde/malondialdehyde modified protein adducts [14–18], liver specific protein (LSP) [19,20], and liver membrane protein (LMA) [21,22], all of which have been shown to play a role in alcohol liver disease. The ability of these chemicals or proteins to modify cell surface membranes or cellular material under the right conditions could help break tolerance and initiate an autoimmune response. Circulating autoantibodies specific for liver proteins or antibodies to adducts on proteins could trigger antibody-dependent cell medicated cytotoxicity, killing or severely damaging hepatocytes, and causing increased inflammation and eventually liver failure. This has been demonstrated using hydroxyl ethyl radical-CYP2E1 adducts on the surface of isolated hepatocytes exposed to ethanol in the presence of normal human peripheral blood mononuclear cells [23–26]. Clearly, the sustained ingestion of alcohol in most people appears to result in the development of an array of autoantibodies to cellular proteins potentially initiating a T-cytotoxic response to hepatocytes [23,27,28].

The finding of circulating antibodies specific for alcohol metabolites indicates there may be a cellular component involved. A report by Terabayashi; et al. supports the concept that cytotoxic T lymphocytes can be generated that recognize syngeneic cells modified with acetaldehyde [28]. In studies consistent with findings reported in this manuscript, malondialdehyde–acetaldehyde modified proteins have been shown to elicit T-cell proliferative responses through antigen processing and presentation by macrophages and dendritic cells [29]. Therefore, if these cellular proteins are available for aldehyde binding and the right conditions are met to produce these adducts, the potential for modification of self proteins or cells exists. These self protein adducts could potentially induce an autoimmune disease mediated by antibodies, $CD4^+$ T-cells, antigen presenting cells, and/or by antibody-dependent cell mediated cytotoxicity.

Current work in this laboratory has shown that the *in vivo* metabolism of alcohol generates the reaction of malondialdehyde (MDA) and acetaldehyde (AA) to form a unique adduct, that has been designated as MAA [18]. These MAA adducts have been shown to elicit antibody and T-cell responses in the absence of adjuvants [29,30]. However, these studies have all utilized soluble proteins and no data is available with regard to the immune response to MAA-modified cells. Therefore, the demonstration that MAA-modified cells can induce T-cell responses would suggest a possible mechanism for alcohol-induced liver damage. Therefore, it was the purpose of this study to determine if MAA adduction of tumor cells can elicit an immune response that may serve as a model system of MHC class I restricted immune response in ALD.

2. Materials and methods

2.1. Mice

Syngeneic DBA/2 mice were obtained from the National Cancer Institute via an interagency agreement with the Department of Veterans Affairs and maintained on water and laboratory chow *ad libitum*. The mice were monitored and determined to be pathogen-free. All procedures were approved by the animal subcommittee of the Omaha VA Medical Center (accredited by American Association for the Accreditation of Laboratory Animal AAALAC) and were in accordance with guidelines of the National Institutes of Health (1985).

2.2. Cell line

The DBA/2 P815 mastocytoma cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in RPMI 1640 medium supplemented with 10% fetal calf, 25 mM HEPES, 2 mM L-glutamine, gentamycin (100 U/ml), and incubated at 37 °C in the presences of 5% CO_2.

2.3. Chemicals and proteins

Bovine serum albumin (Alb) was purchased from Cal Biochem (La Jolla, CA). Acetaldehyde (AA) was obtained from Aldrich Chemical