Available online at www.sciencedirect.com
SCIENCE DIRECT
Clinical Immunology 114 (2005) 307–319
Clinical IMMUNOLOGY
www.elsevier.com/locate/yclim

Functionally divergent T lymphocyte responses induced by modification of a self-peptide from a tumor-associated antigen

Allan D. Hess*, Christopher J. Thoburn, Yuji Miura, Emilie C. Bright

The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University, 1650 Orleans Street, Room 489, Baltimore, MD 21231, USA

Received 3 September 2004; accepted 12 November 2004
Available online 16 December 2004

Abstract

The N- and C-terminal flanking domains of the invariant chain peptide, CLIP, have remarkable immunological properties. Addition of these flanking domains to a foreign peptide antigen increases its immunologic potency. The present studies evaluated whether altering a peptide ligand from the tumor-associated antigen c-neu with the flanking domains of CLIP could modify the systemic immune response. The results indicate that the immunogenicity of an MHC class II restricted peptide (NEU) derived from c-neu was significantly altered by addition of the flanking domains from CLIP. Interestingly, selective modification of the peptide with either the N- or the C-terminal flanking domains resulted in functionally divergent systemic immune responses. Immunization of normal F344 rats with the NEU peptide modified with the N-terminal domain of CLIP (N-NEU) resulted in an immune response primarily consisting of type 1 (IL-2, IFNγ) cytokine producing T cells. On the other hand, type 2 (IL-4) cytokine responses were largely predominant following immunization with the self-peptide modified with the C-terminal flanking domain (NEU-C). The functionally divergent responses elicited by the modified self-peptides were accompanied by significant changes in the expression of the CD28/CTLA4/B7 family of co-stimulatory molecules. Immunization with the N-NEU peptide led to enhanced expression of CD28 in the antigen-specific, CD4+ T cell compartment while expression of B7.1 was dramatically reduced in antigen-specific CD8+ T cells. Comparatively, expression of CTLA4 was down-regulated in the antigen-specific CD4+ T cell compartment following immunization with NEU-C peptide. The N-NEU peptide also had a direct effect on dendritic cells leading to the up-regulation of B7.1 expression. Taken together, functionally divergent systemic immune responses can be elicited by strategically altering a self-peptide ligand with the N- and C-terminal flanking domains of CLIP. Moreover, changes in expression of co-stimulatory molecules that are required for T cell activation and T cell–T cell communication may account for the polarization of the immune response elicited by the chimeric peptides.

Keywords: Self-antigen; Tumor-associated self-antigen; Peptide modification; T cell repertoire diversity; Cytokine polarization; MHC class II; Invariant chain peptide; CLIP; Co-stimulatory receptors; C-neu

Introduction

Presentation of peptide antigens by major histocompatibility complex (MHC) class II molecules on antigen-presenting cells (APCs) plays a pivotal role in the induction of an immune response [1]. While peptides derived from self-proteins bound to MHC class II molecules can potentially activate autoreactive T cells, the immune system is usually functionally tolerant to self-antigens. High-affinity autoreactive cells are deleted in the thymus while peripheral regulatory cells are thought to suppress the activation of autoreactive T cells that escape clonal deletion [2–6]. Although the mechanisms governing nonresponsiveness to self-antigens usually prevent the development of systemic autoimmunity, they also dampen the ability to mount an effective immune response to tumor-associated (TA) antigens derived from "self" proteins [7,8]. Although tolerance to

Abbreviations: TA, tumor associated; MHC, major histocompatibility complex; CLIP, MHC Class II invariant chain peptide; PCR, polymerase chain reaction; CDR3, complementarity-determining region 3; TcR, T cell receptor.

* Corresponding author. Fax: +1 410 502 7163.
E-mail address: adhess@jhmi.edu (A.D. Hess).

1521-6616/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.clim.2004.11.004

the immunodominant epitopes of TA antigens appears to be firmly established, weakly immunogenic cryptic epitopes can stimulate T cells with low avidity clonotypic receptors for the peptide–MHC antigen complex [9,10]. Strategies to augment the overall immune responses to these weak TA antigens have marginal success with detectable increases in the frequencies of antigen-specific cytolytic T cells; however, tumor progression still occurs [10–12]. The paucity of potent MHC class II dependent antigens capable of providing sufficient T cell help may account for the inadequate immune response [13,14].

Recent studies indicate that MHC class II restricted immune responses can be heightened by peptide modification, by increasing surface density of the epitope or by targeting the peptide through the invariant (Ii) chain–MHC class II biosynthesis pathway [15–17]. The Ii chain protein after shepherding the biosynthesis of MHC class II molecules is proteolyzed leaving the peptide termed CLIP within the peptide-binding groove of MHC class II. X-ray crystallographic studies of the MHC class II-peptide complex reveals that CLIP consists of three domains, an MHC class II binding domain and the N- and C-terminal flanking domains that extend outside the peptide binding grove of MHC class II [18,19]. CLIP has remarkable immunologic properties and plays a critical role in MHC class II stabilization, peptide antigen exchange, and antigen presentation [20]. Interestingly, the immunogenicity of MHC class II binding peptide antigens can be significantly enhanced by the addition of the N-terminal flanking domain sequence [21–24]. Recent studies suggest that chimeric constructs of a TA antigen peptide containing the N-terminal flanking domain can induce protective anti-tumor immunity with the preferential activation of type 1 cytokine-producing T helper cells [21]. Although a potentially attractive strategy for immunotherapy, the characteristics of the response may be dependent on the specific modification of the peptide. Chimeric modification of a self-peptide with the C-terminal sequence can lead to the activation of a type 2 cytokine response cytokines and the induction of regulatory T cell activity [25–27]. Although this approach may have potential advantages in modifying the severity of autoimmune disease (by changing the orientation of the systemic immune response to self-antigens), augmenting type 2 cytokine responses in tumor-bearing hosts may be deleterious.

The present studies examined the systemic effect of immunization with a modified self-peptide antigen. Functionally divergent immune responses could be elicited by strategically altering the self-peptide ligand with the flanking domains of CLIP. Examining antigen-specific cells within the CD4 and CD8 compartments revealed that the immunization altered the balance of cytokine expression. Surprisingly, the expression of co-stimulatory molecules was altered and may explain not only the polarization of the systemic response, but also the potent anti-tumor response elicited by the immunization with the chimeric peptide ligands.

Materials and methods

Animals and immunization

Female Fischer (F344) strain rats 4–6 weeks of age were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA). The animals were kept in sterile microisolator cages and fed food and water ad libitum. The animals were immunized intradermally with peptide-loaded dendritic cells (see below) at four distinct sites (5×10^4 cells per site) and repeated 14 days later as described previously [21].

Tumor cells

The mammary adenocarcinoma cancer cell line (CRL 1666, derived from F344 strain rats, American Type Culture Collection (ATCC, Rockville, MD)] was maintained in vitro in McCoy's 5A tissue culture medium (Grand Island Biological Co., Gibco BRL, Grand Island, NY) supplemented with fetal calf serum. The tumor cells express MHC class I and II determinants and c-neu as previously described [21].

Lymphocyte and dendritic cell isolation

Spleens harvested at 12–14 days following immunization were passed through a wire mesh screen to obtain a single cell suspension and the lymphocytes isolated by Ficoll-Hypaque density centrifugation [25,26]. Dendritic cells were isolated from spleen by differential plastic adherence [25,26]. Briefly, spleen cells were incubated for 2 h in tissue culture flasks; the flasks were rinsed thoroughly and incubated for 18 h in RPMI 1640 tissue culture medium supplemented with 10% fetal calf or normal rat serum. Following incubation, the dendritic cells were harvested and washed in tissue culture medium. Expression of OX62 (rat dendritic cell marker, Pharmingen, San Diego, CA) and potent stimulatory activity in a mixed lymphocyte response confirmed dendritic cell identity.

Soluble MHC Class II–Ig fusion product

A soluble rat MHC class II–mouse Ig fusion product (sMHC class II–Ig) that maintains the peptide-binding domain of MHC class II was constructed as previously described [25,26]. Briefly, the extracellular domains of the MHC class II α and β genes ($RT1^l$; expressed on Lewis and F344strain rats) were fused to mouse immunoglobulin γ and κ chains, respectively. The genes encoding the soluble chimeric sMHC class II–Ig molecule were cloned into the pFastBac/Dual vector and the Bac-to-Bac Baculovirus/Sf9 Expression System (Life Technologies, Gaithersburg, MD) used to express the molecular construct. The sMHC class II–Ig fusion product (purified by size exclusion on a Sephacryl 16/60 G-200 column) was also labeled with the fluoro-