Antibodies to a Conserved Region of HLA Class I Molecules, Capable of Modulating CD8 T Cell-mediated Function, Are Present in Pooled Normal Immunoglobulin for Therapeutic Use

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Abstract

Intravenous immunoglobulin (IVIg) is increasingly used for the treatment of autoimmune diseases and the prevention of infections and of graft versus host reactions in recipients of allogeneic bone marrow transplants. The immunomodulatory effects of IVIg are largely dependent on their ability to interact with membrane molecules of lymphocytes. We report here that IVIg recognizes the B07.75-84 peptide, corresponding to a conserved region of the α1 helix of the first domain of HLA-B7 01, which represents a nonpolymorphic determinant of HLA class I molecules. Intact IVIg and its F(ab′)2 fragments bound to the peptide as well as to purified soluble HLA and to HLA on a human T cell line. Binding of IVIg to HLA was assessed by ELISA, immunofluorescence, and real-time analysis of the interaction using the BIAlite system. The binding of antipeptide antibodies to HLA was inhibited by free peptide. Antipeptide antibodies isolated from IVIg by affinity chromatography inhibited CD8 cell-mediated cytotoxicity of an influenza virus-specific human T cell line. The presence in IVIg of antibodies to critical regions of HLA class I molecules suggests a possible role for IVIg in modulation of class I-restricted cellular interactions in the immune response. (J. Clin. Invest. 1996. 97:865–869.) Key words: HLA • IVIg • immunopathology • autoimmunity • immunotherapy

Introduction

In addition to its use as substitutive therapy for primary and secondary antibody deficiencies, intravenous immunoglobulin (IVIg)† has increasingly been used in patients with autoimmune and systemic inflammatory diseases (1). IVIg was also shown to reduce the occurrence and severity of graft versus host disease in recipients of allogeneic bone marrow transplants (2–4). The immunomodulatory effects of IVIg are strongly dependent on the ability of variable regions of therapeutic immunoglobulin to interact with soluble and membrane-associated molecules of the immune system (5). Thus, IVIg has been shown to contain antibodies to idiotypes of autoimmune antibodies (6–8), framework, and idiotypic determinants of the T cell receptor (9), cytokines (10), CD5 (11), and CD4 (12).

MHC molecules are essential in the immune response by displaying antigenic peptides to T cells (13). Class I molecules consist of an MHC-encoded polymorphic α glycoprotein heavy chain of 44-kD and the invariant β2 microglobulin chain. Analysis of the crystal structure of class I molecules has shown that the antigenic peptide-binding site is formed by two homologous α1 and α2 segments of ~90 amino acids at the amino terminus of the α chain. Synthetic peptides derived from conserved regions of the α1 helix have been shown recently to block the differentiation of human CTL precursors in vitro in a non–allele restricted fashion (14) and to induce the permanent acceptance of heart allografts in rats when used in combination with a suboptimal dose of cyclosporin (15).

In the present study, we demonstrate that IVIg contains antibodies capable of recognizing a 10-amino acid peptide derived from a highly conserved portion of the α1 helix of human class I molecules. After isolation from IVIg by affinity chromatography, the antibodies were shown to bind to soluble and membrane-associated HLA class I antigens and to inhibit class I–restricted T cell–mediated cytotoxicity. Antibodies to conserved functional regions of HLA molecules may be relevant for the immunoregulatory effects of IVIg in autoimmunity and transplantation.

Methods

Sources of immunoglobulins. IVIg (Sandoglobulin®) was a gift of the Central Laboratory of the Swiss Red Cross (Bern, Switzerland). F(ab′)2; fragments were prepared from IVIg by pepsin digestion (2.0% wt/wt) (Sigma Chemical Co., St. Louis, MO) in acetate buffer, pH 4.1, for 18 h at 37°C and chromatography on protein A Sepharose. F(ab′)2; fragments were free of IgG and Fc fragments as assessed by SDS-PAGE and ELISA. The human IgG myeloma was a gift from Dr. D. Hurez (Angers, France).

Mouse hybridoma W6/32HL producing mAb that recognizes a nonpolymorphic determinant of the α chain of human HLA-A, B, and C molecules, was obtained from the European Collection of Animal Cell Cultures (Salisbury, UK). The antibody was used to affinity

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purify HLA class I molecules from lysates of platelets of 30 unselected healthy blood donors.

Peptides. The B07.75-84 peptide (RESLRNLRGY) corresponding to residues 75-84 of the α1 helix of the HLA B7 molecule and the irrelevant peptide RYERNLLARI were synthesized as described (15). The peptides contain a similar number of charged amino acids.

Antipeptide antibodies. For affinity purification of antipeptide antibodies from IVIg, the B07.75-84 peptide was coupled to activated CH Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). F(ab'2) fragments of IVIg in PBS were interacted with the affinity matrix overnight at 4°C before eluting bound antibodies using 0.2 M glycine-HCl buffer, pH 2.8. The eluate was immediately neutralized with 4.0 M Tris, concentrated using a Centricon-30 concentrator (Amicon Inc., Beverly, MA) and dialyzed against PBS.

The binding of antipeptide antibodies to HLA was assessed by ELISA. Briefly, glutaraldehyde-pretreated or untreated MaxiSorp Immuno-Plates (Nunc, Denmark) were coated with the B07.75-84 peptide or with HLA-enriched antigen obtained from platelet lysates, blocked by incubation with 1.0% gelatin in PBS (PBS-G), washed, and incubated for 2 h at room temperature with serial dilutions of the antibodies to be tested. Bound antibodies were revealed using peroxidase-conjugated anti–human F(ab’2) antibodies (Jackson Immunoresearch Laboratories, West Grove, PA). The optical density was determined at 490 nm using an Emax ELISA system (Pharmacia) that allows real-time analysis.
Affinity-purified antipeptide antibodies from IVIg were used for indirect immunofluorescence staining of HPB-ALL cells. As shown in Fig. 2, the mean fluorescence intensity of cells stained with IgG and F(\(ab'\))\(_2\) fragments eluted from the B07.75-84 column was significantly higher than that of cells stained with F(\(ab'\))\(_2\) fragments of IVIg in the effluent of the peptide affinity column (\(P < 0.01\); Kolmogorov-Smirnov test). Further, intact IgG eluted from the B07.75-84 affinity column was capable of inhibiting the binding of fluoresceinated W6/32HL to HPB-ALL cells (data not shown). Thus, anti-B07.75-84 peptide antibodies that are present in IVIg recognize cell surface HLA molecules in addition to the soluble form of the molecule and the free peptide.

The formation of noncovalent complex between IVIg and HLA molecules was measured in real time using the BIAlextm biosensor analysis system. Fig. 3 shows an overlay plot of the sensorgrams obtained with the injections of four concentrations of F(\(ab'\))\(_2\) fragments of IVIg ranging from 150 to 750 \(\mu\)g/ml. As shown in the figure, the number of F(\(ab'\))\(_2\) fragments of IVIg molecules binding to the antigen increased during the first 360 s and then decreased for the next 150 s. At 300 \(\mu\)g/ml of antipeptide antibodies, 170 R.U. were retained on the surface of B07.75-84. The kinetics of dissociation of IVIg from sensor chip–bound B07.75-84 was evaluated. The apparent dissociation rate constant (\(K_d\)) that measures the amount of anti-B07.75-84/B07.75-84 complexes that decay per second was then calculated. The overall affinity of binding was found to be 10\(^{-8}\) M. The specificity of the complex formation as assessed by real-time analysis was confirmed by the ability of soluble HLA class I molecules to inhibit the binding of antipeptide antibodies to the immobilized peptide (data not shown).

Inhibition of class I–restricted T cell–mediated cytotoxicity. The biological relevance of anti-B07.75-84 peptide antibodies was examined in a cell-mediated cytotoxicity assay using EBV-transformed B cells sensitized with the M.58-66 influenza virus peptide as target cells and a peptide-specific CD8\(^+\) T cell line as a source of effector cells. Affinity-purified anti-B07.75-84 peptide antibodies inhibited cytotoxicity in a dose-dependent manner (Fig. 4).

**Discussion**

In the present study, we demonstrate that a subpopulation of antibodies present in normal IgG for therapeutic use (IVIg) binds to a peptide (B07.75-84) corresponding to residues 75-84 of the \(\alpha_1\) helix of the first domain of HLA-B7 01 which repre-
sents a nonpolymorphic and highly conserved determinant of HLA class I molecules. Only 10 variations have been found in this region among more than 85 HLA-A, -B, and -C alleles (17). The sequence is likely to be involved in the interaction of class I molecules with the T cell receptor (13). The B07.75-84 peptide blocks the differentiation of human cytotoxic T lymphocyte precursors in vitro (14). The peptide was shown to induce the permanent acceptance of heart allografts in rats when used in combination with cyclosporin (15, 18).

We have demonstrated the reactivity of affinity-purified antibodies to the B07.75-84 peptide present in IVIg with HLA molecules isolated from human platelets by ELISA and by real-time analysis of complex formation using the BIAalitTM system. Specificity of the binding was shown by the ability of free peptide to inhibit the binding reaction. The biological relevance of the antibodies was demonstrated by the ability of anti-peptide antibodies isolated from IVIg to inhibit class I–restricted cytotoxicity of human CD8+ T cells specific for a peptide of influenza virus.

IVIg is increasingly used in the treatment of certain autoimmune diseases (1). IVIg exhibits immunomodulatory effects in diseases mediated by autoantibodies and in diseases believed to be primarily mediated by autoaggressive T cells, in the human and in experimental animals (5). IVIg is routinely used for the prevention of graft-versus-host reaction in recipients of allogeneic bone marrow transplants (3). Recent evidence indicates that IVIg may modulate the generation of anti–class I antibodies in hyperimmunized hemodialyzed patients awaiting a renal transplant (19). A possible mechanism underlying the latter effect involves the induction of antidiotypic antibodies directed against anti-class I antibodies present in IVIg (20).

In vitro modulation of T cell activity by IVIg has been documented previously (21, 22). IVIg has been shown to inhibit the proliferation of T cells stimulated with mitogens, anti-CD3 antibodies, tetanus toxoid antigen, or ionophore and phorbol esters, as well as of non–T cell lines, by variable region–dependent mechanisms which are as yet not fully understood (21, 23). It has also been shown that anti-staphylococcal toxin antibodies present in IVIg inhibit the proliferative response of T cells to staphylococcal enterotoxin (24). Since stimulation of T cells via superantigens involves MHC class II molecules, antibodies directed against class II antigens or mere presence of soluble class II molecules in IVIg, could also contribute to inhibition of the proliferation of T cells stimulated with staphylococcal enterotoxin B (SEB) (25).

The diversity of variable regions present in IVIg preparations is essential for the ability of therapeutic Ig to interact with antibodies and cells and to select immune repertoires (5). Since IVIg is prepared from large pools of plasma of healthy donors, it may be considered as containing the spectrum of antibody specificities expressed within the normal IgG antibody repertoire. Natural antibodies of the IgG isotype directed against self components are present in normal human serum (26–30). IVIg has been shown to contain antibodies against several surface molecules of homologous lymphocytes involved in immunoregulation, including CD5 (11), CD4 (12), idiotypes of Igs (7), as well as framework and clonotypic determinants of human T cell receptor (9). In addition, soluble forms of CD4, CD8, class I, and class II molecules have been identified in IVIg (25, 31). Several of the molecules that are reactive with IVIg have been the targets of immunomodulatory therapy with monoclonal or genetically engineered antibodies and/or targets of immunomanipulation for tolerance induction to self and to allogeneic determinants in experimental models (32–36).

The presence in therapeutic Ig preparations of antibodies directed against a highly conserved region of HLA class I antigens endowed with immunoregulatory properties provides a basis for immunomodulation of class I–restricted cellular interactions in the immune response by IVIg.

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References


