Introduction
Proteins are the most abundant biological macromolecules in the living systems and serve crucial functions in essentially all biological processes (Richardson, 1981). All proteins, whether from the most ancient lines of bacteria or from the most complex forms of life, are constructed from the same ubiquitous set of 20 amino acids, covalently linked in characteristic linear sequences. Moreover, proteins exhibit enormous diversity of biological functions and are the most important molecular instruments through which genetic information is expressed. They function as catalyst, transport and store other molecules such as oxygen, provide mechanical support and immune protection, generate movement, transmit nerve impulses and control growth and differentiation (Creighton, 1993).

**Immune system**

All vertebrates have an immune system capable of distinguishing molecular “self” from “nonself” and then destroying those entities identified as nonself (Blom et al., 1998). Broadly speaking, immune system has been divided into two categories—innate and adaptive immune systems.

(i) **Innate immune system** provides the first line of defense against infection. Most components of innate immunity are present before the onset of infection and constitute a set of disease-resistance mechanisms that are not specific to a particular pathogen i.e. they exhibit non-specific recognition of infectious agent.

(ii) **Adaptive immune system** consists of immunoglobulins and lymphocytes. It is highly specific for a particular antigen and remembers the infectious agent and can prevent it from causing the disease later. Thus, the two key features of adaptive immune response are specificity and memory.

The immune response consists of two complementary systems, the cellular and humoral immune response (Goldsby et al., 2000). In the humoral immune response, soluble proteins called antibodies (immunoglobulins) function as recognition elements that bind to foreign molecules and serve as markers signaling foreign invasion. Antibodies are secreted by plasma cells, which are derived from B lymphocytes (B cells). In the cellular immune response, cells called cytotoxic T lymphocytes (also commonly called killer T cells) kill cells that display foreign
motifs on their surfaces. Another class of T cells called helper T lymphocytes contributes to both the humoral and the cellular immune responses by stimulating the differentiation and proliferation of appropriate B cells and cytotoxic T cells. The cellular immune response is mediated by specific receptors that are expressed on the surfaces of the T cells (Abbas et al., 1994).

**Immunoglobulins**

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and function as antibodies. The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field. Immunoglobulins are present in the serum and tissue fluids of all mammals (Burton., 1990).

**Basic structure of immunoglobulins**

The fundamental structure of immunoglobulins was first established by Gerald Edelman and Rodney Porter. Although different immunoglobulins can differ structurally, however, they all are built from the same basic units (Fig. 1) (Frazer and Capra., 1999; Wang et al., 2006).

**a) Heavy and light chains**

All immunoglobulins have a four chain structure as their basic unit (MW ~ 150,000 daltons). They are composed of two identical light chains (MW ~ 25,000 daltons) and two identical heavy chains (50,000 daltons or more).

**b) Disulfide bonds**

(i) *Inter-chain disulfide bonds* - The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions (Davies and Chacko., 1993). The number of inter-chain disulfide bonds varies among different immunoglobulin molecules.

(ii) *Intra-chain disulfide bonds* - Within each of the polypeptide chains there are also intra-chain disulfide bonds.
Fig. 1  Schematic drawing of the basic structure of the human immunoglobulin. The amino-terminal end is characterized by sequence variability (V) in both the heavy and light chains, referred to as the $V_H$ and $V_L$ regions respectively. The rest of the molecule has a relatively constant (C) structure. The constant portion of the heavy chain is further divided into three structurally discrete regions: $C_H^1$, $C_H^2$ and $C_H^3$. Carbohydrate groups are attached to the $C_H^2$ domains of the heavy chains.

Source:  (Burton., 1990)
c) Variable (V) and constant (C) regions

Both the heavy and light chains are divided into two regions based on variability in the amino acid sequences. These are the:

(i) Light chain - $V_L$ (110 amino acids) and $C_L$ (110 amino acids)
(ii) Heavy chain - $V_H$ (110 amino acids) and $C_H$ (330-440 amino acids)

d) Hinge Region

This is the region at which the arms of the antibody molecule form a ‘Y’. It is called the hinge region because there is some flexibility in the molecule at this point.

e) Domains

Three dimensional images of the immunoglobulin molecule show that it is not straight, rather it is folded into globular regions each of which contains an intra-chain disulfide bond. These regions are called domains.

(i) Light chain domains - $V_L$ and $C_L$
(ii) Heavy chain domains - $V_H$, $C_H1 - C_H3$ (or $C_H4$)

X-ray crystallographic analysis revealed that these domains are folded into a characteristic compact structure called the ‘immunoglobulin fold’.

f) Oligosaccharides

Carbohydrates are attached to the $C_H2$ domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.

Immunoglobulin classes

The immunoglobulins can be divided into five different classes, based on differences in the amino acid sequences in the constant region of the heavy chains (Kolar and Capra., 2003). They differ in size, charge, amino acid composition and carbohydrate content (Davies and Metzger., 1983; Turner., 1977). The physicochemical properties of the immunoglobulins are summarized in Table 1.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IgG</th>
<th>IgM</th>
<th>IgA</th>
<th>IgD</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heavy chain</strong></td>
<td>γ</td>
<td>μ</td>
<td>α</td>
<td>δ</td>
<td>ε</td>
</tr>
<tr>
<td><strong>Heavy chain sub-classes</strong></td>
<td>γ₁, γ₂, γ₃, γ₄</td>
<td>None</td>
<td>α₁, α₂</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td><strong>Light chain</strong></td>
<td>κ or λ</td>
<td>κ or λ</td>
<td>κ or λ</td>
<td>κ or λ</td>
<td>κ or λ</td>
</tr>
<tr>
<td><strong>Molecular formula</strong></td>
<td>γ₂κ₂</td>
<td>(μ₂λ₂)ₙ</td>
<td>(α₂κ₂)ₙ</td>
<td>δ₂κ₂</td>
<td>ε₂κ₂</td>
</tr>
<tr>
<td>Molecular form</td>
<td>-</td>
<td>Pentamer</td>
<td>Mostly dimer</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Amount in Ig pool (%)</strong></td>
<td>70-75</td>
<td>~10</td>
<td>15-20</td>
<td>~1</td>
<td>very scarce</td>
</tr>
<tr>
<td><strong>Molecular weight</strong></td>
<td>150,000</td>
<td>900,000</td>
<td>160,000/385,000</td>
<td>180,000</td>
<td>190,000</td>
</tr>
<tr>
<td><strong>Mean serum conc. (mg/ml)</strong></td>
<td>0.5-9.0</td>
<td>1.5</td>
<td>0.05-3.0</td>
<td>0.03</td>
<td>0.00005</td>
</tr>
<tr>
<td><strong>Sedimentation coefficient</strong></td>
<td>7S</td>
<td>19S</td>
<td>7S, 11S</td>
<td>7S</td>
<td>8S</td>
</tr>
<tr>
<td><strong>Intravascular distribution (%)</strong></td>
<td>45</td>
<td>80</td>
<td>42</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td><strong>Carbohydrate content (%)</strong></td>
<td>2-3</td>
<td>12</td>
<td>7-11</td>
<td>9-14</td>
<td>12</td>
</tr>
</tbody>
</table>

Source: (Roitt et al., 1996)
Immunoglobulin subclasses

The classes of immunoglobulins can be divided into subclasses based on small differences in the amino acid sequences in the constant region of the heavy chains (Shakib., 1990). Some antigenic differences between the heavy chains, γ and α, give rise to different subclasses in IgG and IgA respectively. Moreover, none of the subclasses have been described for IgM, IgD or IgE.

1. IgG Subclasses

   (i) IgG1 - Gamma 1 heavy chains
   (ii) IgG2 - Gamma 2 heavy chains
   (iii) IgG3 - Gamma 3 heavy chains
   (iv) IgG4 - Gamma 4 heavy chains

2. IgA Subclasses

   (i) IgA1 - Alpha 1 heavy chains
   (ii) IgA2 - Alpha 2 heavy chains

Antigenic determinants/ epitopes on immunoglobulins

Immune cells do not interact with, or recognize, an entire immunogen molecule; instead, lymphocytes recognize discrete sites on the macromolecule called epitopes, or antigenic determinants. Epitopes are the immunologically active regions of immunogen that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodies (Wilson and Stanfield., 1994). The antigenic determinants, or epitopes, on immunoglobulin molecules fall into three major categories: isotypic, allotypic, and idiotypic determinants (Playfair., 1992).

a) *Isotype:* Isotypic determinants are constant-region determinants and each isotype is encoded by a separate constant region gene. All members of a species carry the same constant-region genes. Different species inherit different constant-region genes and therefore express different isotypes. Therefore, when an antibody from one species is injected into another species, the isotypic determinants will be
recognized as foreign, inducing an antibody response to the isotypic determinants on the foreign antibody.

b) **Allotype:** Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the genes. These alleles encode subtle amino acid differences, called allotypic determinants, which occur in some, but not all, members of a species. The sum of the individual allotypic determinants displayed by an antibody determines its ‘allotype’. In humans, allotypes have been characterized for all four IgG subclasses, for one IgA subclass, and for the κ light chain. Antibody to allotypic determinants can be produced by injecting antibodies from one member of a species into another member of the same species who carries different allotypic determinants.

c) **Idiotype:** The unique amino acid sequence of the variable region of a given antibody can function not only as an antigen-binding site but also as a set of antigenic determinants. The idiotypic determinants arise from the sequence of the heavy- and light-chain variable regions. Each individual antigenic determinant of the variable region is referred to as an idiotope. In some cases an idiotope may be the actual antigen-binding site, and in some cases an idiotope may comprise variable-region sequences outside of the antigen binding site. Each antibody will present multiple idiotopes and the sum of the individual idiotopes is called the ‘idiotype’ of the antibody. Anti-idiotype antibody is produced by injecting antibodies that have minimal variation in their isotypes and allotypes, so that the idiotypic difference can be recognized.

**General functions of immunoglobulins**

Immunoglobulins are central molecular players in the immune response. They perform two important functions in the biological system-

a) **Antigen binding:** Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host. Each immunoglobulin actually binds to a specific antigenic determinant. The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all antibodies is at least two and in some instances more.
b) **Effectors functions:** The immunoglobulins mediate a variety of the effector functions (Berzofsky and Berkower., 1999). Such effector functions include:

(i) *Fixation of complement* - This results in lysis of cells and release of biologically active molecules.

(ii) *Binding to various cell types* - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts, which results in transfer of the immunoglobulin across the placenta. As a result, the transferred maternal antibodies provide immunity to the foetus and newborn.

**Immunoglobulin G**

1. **Structural properties:**

The IgG molecule may be thought of as a ‘typical’ antibody. It is a monomer having four inter-chain disulphide bonds- two connecting the heavy chains at the hinge region and the other two connecting the two light chains to the heavy chains (Janeway *et al.*, 2001) (Fig. 2). IgG molecule also has four intra-chain disulphide bonds, residing in each domain of the heavy and light chains, stabilizing these domains. The intrachain disulphide bonds in V\textsubscript{H} and V\textsubscript{L} regions are required in functional antigen binding (Glockshuber *et al.*, 1992). Each disulphide bond encloses a peptide loop of 60-70 amino acid residues; if the amino acid sequences of these loops are compared a striking degree of homology is revealed (Kolar and Capra., 2003). Essentially this means that each immunoglobulin peptide chain is composed of a series of globular regions with every similar secondary and tertiary structure (folding).

2. **Functional properties:**

IgG is the most versatile immunoglobulin (Ig) because it is capable of carrying out all of the functions of immunoglobulin molecules.
**Fig. 2** Spacefill model of the crystal structure of an intact human IgG1.

**Source:** (Kolar and Capra., 2003)
a) IgG is the major Ig in serum - 75% of serum Ig is IgG
b) IgG is the major Ig in extra vascular space.
c) IgG is the major antibody class of the secondary response and is usually of higher affinity than IgM, which dominates in the primary response.
d) **Placental transfer** - IgG is the only class of Ig that crosses the placenta. Maternal IgG confers immunity in neonates. Transfer is mediated by a receptor on placental cells for the Fc region of IgG (Van de Winkel and Capel., 1993). In man, IgG molecules of all subclasses cross the placenta and confer a high degree of passive immunity to the newborn.
e) **Complement fixation** - IgG (and IgM) can fix complement by activating the C3 protein at the end of the complement cascade allowing ADCC (antibody dependent cell-mediated cytotoxicity) to occur.
f) **Binding to cells** - Macrophages, monocytes, PMNs (polymorphonuclear leukocytes) and some lymphocytes have Fc receptors for the Fc region of IgG. IgG is a good opsonin. Binding of IgG to Fc receptors on other types of cells results in the activation of other functions.

**IgG subclasses**

The four subclasses of human IgG differ only slightly in their amino acid sequences (Shakib and Stanworth., 1980). Most of the differences are clustered in the hinge region and give rise to differing patterns of interchain disulphide bonds between the four proteins (Fig. 3). The IgG subclasses display a 95% sequence homology, with the major differences located in the hinge region (Table 2). The hinge region provides flexibility to IgG molecule. The flexibility of human IgG decreases in the order IgG3 > IgG1 > IgG4 > IgG2. The observed variations in the hinge properties are of great functional importance as they allow IgG to cope efficiently with different spacings and orientations of antigenic epitopes. The most striking structural difference is the elongated hinge region of IgG3 which accounts for its higher molecular weight and possibly for some of its enhanced biological activity.

It may be noted that the IgG subclasses also differ with respect to their functional properties (Jefferis., 1992). For instance, IgG1 will dominate in an immune response against tetanus toxoid, IgG2 against polysaccharides, IgG3 against rhesus-D
Fig. 3  General structure of the four subclasses of human IgG, which differ in the number and arrangement of the interchain disulphide bonds (yellow portion) linking the heavy chains. A notable feature of human IgG3 is its more interchain disulphide bonds.

Source: (Ollmann-Saphire et al., 2002)
**TABLE 2**

**Structural and physicochemical properties of the human immunoglobulin G subclasses**

<table>
<thead>
<tr>
<th>Properties</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heavy chain type</strong></td>
<td>γ 1</td>
<td>γ 2</td>
<td>γ 3</td>
<td>γ 4</td>
</tr>
<tr>
<td><strong>% of IgG in human serum</strong></td>
<td>66</td>
<td>23</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td><strong>Mol wt (x 10^3)</strong></td>
<td>146</td>
<td>146</td>
<td>170</td>
<td>146</td>
</tr>
<tr>
<td><strong>No of inter H chain disulphide bonds</strong></td>
<td>2</td>
<td>4</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td><strong>Hinge amino acid number</strong></td>
<td>15</td>
<td>12</td>
<td>62</td>
<td>12</td>
</tr>
<tr>
<td><strong>Half-life (days)</strong></td>
<td>21</td>
<td>20</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td><strong>Mean serum conc. (mg/ml)</strong></td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Source:** (Kuby., 1992)
and IgG4 against factor VIII. IgG1 and IgG3 are efficient complement activators, while IgG2 is effective only at high concentrations, IgG4 is ineffective. The ability of the IgG subclasses to interact with the receptors varies, IgG1 and IgG3 being the most efficient. In contrast to the other immunoglobulins, all four IgG subclasses bind staphylococcal protein A and protein G, except for IgG3, which only binds the latter. The functional properties of the human IgG subclasses have been summarized in Table 3.

**Glycosylation in IgG**

Human IgG is a glycoprotein with 2.8±0.4 mol of oligosaccharide per mol of IgG (Rademacher and Dwek., 1983). Most of the carbohydrate is present on the Fc fragment (2.0 mol/mol of Fc) at a conserved glycosylation site (Asn-297) whereas the remaining sugar occurs at variable positions (\(V_L\) and \(V_H\)) within Fab fragments. Both N- and O-linked carbohydrate moieties have occasionally been found within the variable regions of immunoglobulins (Krapp et al., 2003). The presence of large, bulky carbohydrates on the surface of the variable regions (\(V_L\) and \(V_H\)) contributes to, and probably alters, the conformation of the protein at this site. Therefore, glycosylation in the variable domains often seems to interfere with the antigen-binding properties of these antibodies (Wallik et al., 1988; Wright et al., 1991; Leung et al., 1995). The N-linked carbohydrate structures found in the variable regions seem to differ from those present in the constant regions of IgG (Tachibana et al., 1997). Proper glycosylation is critical for correct functioning of the antibodies (Wright and Morrison., 1997). Furthermore, the sugar composition of oligosaccharides is also critical in antibody functions.

**Immunogenicity of IgG**

For a substance to behave as an immunogen, it should possess the following characteristics- Foreignness, molecular size, chemical nature and heterogeneity. When an immunogen is introduced into an organism, the degree of its immunogenicity depends on the degree of its foreignness. The most active immunogens tend to have a molecular mass of 100,000 daltons. Studies have shown that copolymers composed of different amino acids or sugars are usually more immunogenic than homopolymers of their constituents. These studies show that chemical complexity contributes to
<table>
<thead>
<tr>
<th>Properties</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary response</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Secondary response</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Neutralization</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Opsonization</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Sensitization for killing by NK-cells</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Sensitization of mast cells</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Classical pathway of complement activation</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Alternative pathway of complement activation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Transport over epithelium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Transport over placenta</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Reactivity with staphylococcal protein A</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Reactivity with staphylococcal protein G</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

Source: (Roitt et al., 1996)
immunogenicity. Proteins and polysaccharides are among the most potent immunogens, although nucleic acids and lipids can be relatively immunogenic (Laver et al., 1990).

Immunogenicity is the ability of a protein to induce an immune response in the host body. Furthermore, the ability of a protein to react specifically with the functional binding site of a complementary antibody is known as its antigenic specificity or antigenicity. Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules, called haptens, are antigenic but incapable, by themselves, of inducing a specific immune response. In other words, they lack immunogenicity. Immunogenicity depends on extrinsic factors such as the host immunoglobulin repertoire, self-tolerance, the production of cytokines and various cellular and regulatory mechanisms, definable only in the context of a particular host (Berzofsky., 1985).

Since immunoglobulins are glycoproteins, they can act as an antigen, eliciting an immune response that generates anti-immunoglobulin antibodies. The structural features (antigenic determinants) that define isotypes (IgG, IgM, IgA, IgD and IgE) are immunogenic in different species. For instance, injection of human IgG into a rabbit would generate antibodies against those antigenic determinants that define the IgG isotype. Generally, the greater the phylogenetic distance between the two species, the greater would be the immunogenicity generated. Moreover, there are some exceptions to this rule. Some macromolecules (e.g., collagen and cytochrome c) have been highly conserved throughout evolution and therefore display very little immunogenicity across diverse species lines. Conversely, some self-components (e.g., corneal tissue and sperm) are effectively sequestered from the immune system, so that if these tissues are injected even into the animal from which they originated, they will function as immunogens.

Glycation

Glycation is the non-enzymatic addition of reducing sugars (e.g., glucose, fructose, mannose, etc.) to the biological macromolecules (proteins, nucleic acids and lipids). The free carbonyl groups (-C=O) of the sugar and related moieties react with
the free amino (-NH₂) residues of the macromolecules in a series of chemical processes known as the Maillard reaction. Since it was first described by Maillard, a French chemist, and has thus been named after him (Maillard., 1912). Initially, the carbonyl groups of reducing sugars can readily interact with the free amino groups of a protein to form Schiff base (aldimine), a thermodynamically unstable form. Therefore, the Schiff base undergoes rearrangements through acid-base catalysis to form a more stable early glycation product known as Amadori product (ketoamine) (Neglia et al., 1985). Once formed, an Amadori product can undergo cyclization, dehydration, condensation and oxidation to generate a heterogeneous class of compounds referred to as advanced glycation end products (AGEs), characterized by a wide structural and physicochemical diversity (Ahmed., 2005). Free radicals have been reported to bring about AGE products (Baynes., 1991). AGEs are composed of both fluorescent and non-fluorescent species. Several AGEs structure have been identified including pyrraline (Hayase et al., 1989), pentosidine (Sell and Monnier., 1989), Nε-(carboxymethyl)lysine (CML) (Ahmed et al., 1986), crosslines (Nakamura et al., 1992), imidazole (Konishi et al., 1994; Lo et al., 1994), methylglyoxal lysine dimer (Frye et al., 1998), Nε-(carboxyethyl)lysine (Ahmed et al., 1997), vesperlysine A, B and C (Nakamura et al., 1997), glyoxal lysine dimer (Frye et al., 1998), argpyrimidine (Oya et al., 1999), and carboxymethylarginine (Iijima et al., 2000).

AGEs of DNA are formed spontaneously by the reaction of carbonyl compounds such as sugars, glyoxal, methylglyoxal or dihydroxyacetone in vitro and in vivo. In in vitro studies, mutagenic effects of DNA-AGE such as deletions, insertions and transposon activation were shown in bacterial model systems (Pischetsrieder et al., 1999). Furthermore, it is shown that DNA can be glycated in vitro yielding carboxyethylguanosine as major products. However, little is known about the biological consequences of DNA-AGEs. Moreover, it has been reported that AGEs lead to the irreversible modifications on proteins (McDonald et al., 1979; Schnider and Kohn., 1980; Shaklai et al., 1984; Watkins et al., 1985) and tissues (Sims et al., 1996). AGE-modified proteins such as hemoglobin, immunoglobulin and lipoproteins are involved in the pathological responses observed in rheumatoid arthritis, diabetes mellitus, atherosclerosis, amyloidosis, neurodegenerative disorders, aging, etc (Brownlee., 1995; Bierhaus et al., 1998; Wautier and Guillausseau., 2001; Newkirk et al., 2003).
A critical property of AGEs is their ability to activate receptor for advanced glycation end products (RAGE), a signal transduction receptor of the immunoglobulin superfamily. Due to such interaction, AGEs impart a potent impact in tissues, stimulating processes linked to inflammation and its consequences. Physiological AGE formation from the stable ketoamine glycated protein was observed to correlate with oxidative and inflammatory processes and not with glucose levels (Newkirk et al., 2003).

**Protein glycation**

Glycation is a post-translational chemical reaction that results in nonenzymatic glycosylation at proteins’ exposed amino groups, primarily the alpha amino terminal and the guanidino and epsilon amino groups on the arginine and lysine side chains respectively. Although positively charged primary amines are generally located on the protein structure’s surface, only some of these accessible sites will be specifically reactive toward the reducing sugar molecules. No specific sequence that signals a potential glycation site has been observed; however, three-dimensional local environments that contain histidine residues or basic residues (lysine and arginine) have been observed in several structurally known proteins e.g., liver alcohol dehydrogenase, RNase A, DNase I, albumin and hemoglobin (Shapiro et al., 1980; Watkins et al., 1985; Iberg and Fluckiger., 1986; Shilton and Walton., 1991; Quan et al., 1999; Zhang et al., 2001) to correlate with glycation occurrence. Advanced glycation modified proteins in vivo can induce AGE-specific receptor expression (RAGE), anti-AGE protein immune response and alterations in cellular signaling and function (Ligier et al., 1998; Kislinger et al, 1999; Basta et al, 2002; Virella et al, 2003). Long-term AGE-damaged proteins become crosslinked, structurally altered, fragmented and ultimately aggregated. These non-native states of proteins are toxic for many cell types (e.g., endothelial, neuronal, retinal, leukocytes) and are associated with pathogenesis of many diseases and secondary complications (Bouma et al., 2003).

**IgG glycation**

IgG is a major serum protein, of approximately 1300-amino acid and 150-kD heterodimeric structure, rich in lysine residues and mainly responsible for antigen
clearance (Quan et al., 2008). IgG has a long biological half-life among all plasma proteins (24 days) (Austin et al., 1987). The dominant factor in protein glycation is the half-life of protein; greater the half life, greater the glycation (Austin et al., 1987). So, IgG exhibits maximum glycation in vivo. Glycation of IgG is of special interest due to its influence on the functionality of immunoglobulins and overall immunocompetence especially with regard to their ability to bind antigens and induce the complement cascade (Goodarzi et al., 2004). Previous studies provide convincing evidence that glycation of monoclonal antibodies impairs their functional ability to bind to their respective antigens (Kaneshige., 1987; Sasaki et al., 1993; Kennedy et al., 1994). Dolhofer-Bliesner in a subsequent study reported that complement fixation and protein A binding are markedly impaired by IgG glycation (Dolhofer-Bliesener and Gerbitz., 1990).

In addition to four N-terminal amino acids, human IgG1 has approximately 80 lysine residues, making IgG a susceptible target for glycation. Both the heavy chain and the light chain of IgG can be AGE-modified and it is likely the 60 amino acid hinge region unique to IgG3, which has several lysine and arginine residues, is also glycated. Recently, it has been reported that glycated IgG (AGE-IgG) has a potential role in rheumatoid arthritis (RA) (Ligier et al., 1998; Newkirk et al., 1998; Lucey et al., 2000). It is a target of circulating autoantibodies (anti-AGE-IgG antibodies) in RA patients. (Tai and Newkirk., 2000). These anti-AGE-IgG antibodies prevent the clearance of AGE-IgG by forming immune complexes and thus blocking the recognition of AGEs by specific receptors of the reticuloendothelial system (Bucala and Cerami., 1992; Vlassara., 1997). The anti-AGE-IgG immune response is also associated with the elevated levels of κ and λ light chains which are an integral part of IgG. Interestingly, elevated levels of a heavily glycated κ, in particular, are associated with the presence of the anti-AGE-IgG antibodies. A similarly heavily glycated λ light chain has not been detected which may reflect the two facts: 75% of all light chain genes used are κ and that there are more free amino groups that could be potentially glycated in κ than in λ light chains (Kabat et al., 1991).

**Advanced glycation end products**

The presence of glucose in the human blood continuously supplies the reducing sugar to react with the body proteins in a physiological Maillard reaction
pathway leading to the formation of AGEs. AGEs are characterized by fluorescence, brown colour and inter- and intramolecular cross-linking (Maillard., 1912; Finot., 1982). The study of AGEs represents one of the most promising areas of research today. Although the initial chemistry behind their formation has been known since the early 1900’s, it is only in the last 20 years or so that important work has been done to elaborate it. The chemical processes and pathways that ultimately lead to AGE formation have, however, yet to be fully clarified (John and Lamb., 1993).

Apart from diabetic microvascular disease, AGEs have also been implicated in a wide and seemingly disparate range of pathologies such as connective tissue diseases particularly in rheumatoid arthritis, neurological conditions such as Alzheimer’s disease and end-stage renal disease (ESRD). In vitro work has mostly shown AGEs to be part of complex interactions within oxidative stress and vascular damage, particularly in atherosclerosis. Histopathological studies have shown AGE accumulation in a variety of tissue types including renal cortex, coronary atheroma, amyloid plaques in Alzheimer’s disease, cartilage in rheumatoid arthritis, cardiac muscle, lung and liver.

Apart from their presence in a wide variety of body tissues, AGEs have also been identified from exogenously derived sources such as tobacco (Nicholl and Bacula., 1998) and certain foods, particularly those that are heated (O’Brien and Morrissey., 1989). Temperatures over 120 °C (~248 °F) greatly accelerate the AGE formation, but lower temperatures with longer cooking times also promote their formation. Glycation may also contribute to the formation of acrylamide (Stadler et al., 2002), a potential carcinogen, during cooking. Recent studies have shown that exogenous AGEs are implicated in the initiation of retinal dysfunction, cardiovascular diseases, type II diabetes, and many other age-related chronic diseases. Food manufacturers have added AGEs to foods, especially in the last 50 years, as flavor enhancers and colorants to improve appearance (Peppa et al., 2003). Foods, with significant browning, caramelization or directly added preformed AGE products, are exceptionally rich with these proinflammatory and disease-initiating compounds. A very partial listing of foods with very high exogenous AGEs includes: donuts, barbecued meats, cake, and dark colored soda pop (Koschinsky et al., 1997).
Formation of AGEs

Reducing sugars, such as glucose, react non-enzymatically with amino group of proteins through the Maillard reaction forming AGEs. Of importance in the Maillard reaction is the formation of reactive intermediate products, known as α-dicarbonyls or oxaldehydes (such as 3-deoxyglucosone (3-DG), methylglyoxal (MO), glyoxal) (Wells-Knecht et al., 1996; Skovsted et al., 1998; Baynes and Thorpe., 1999) from all the stages of the glycation process i.e. by degradation of glucose or Schiff’s bases in early glycation, or from Amadori products such as fructosamine in the intermediate stages of glycation (Thornalley et al., 1999). Thus α-oxoladehydes could be considered as important focal points of how glucose can go on to form AGEs by the classical Maillard reaction (Fig. 4). These reactive carbonyl intermediates can lead to the formation of AGEs such as MOLD (methyl glyoxal lysine dimer), DOLD (deoxyglucosone-lysine dimer), CEL (Nε-[carboxyethyl]-lysine) and pyrraline by non-oxidative means (Thornalley et al., 1999). If oxidation accompanies glycation then the products formed are also known as glyoxidation products. The examples of such AGEs are pentosidine and CML (Nε-[carboxymethyl]-lysine) (Bierhaus et al., 1998; Miyata et al., 1997). Pentosidine and CML are the best characterized AGEs. CML could serve as a general bio-marker of oxidative stress resulting from glyoxidation (Fu et al., 1996).

Detection of AGEs

AGE detection is based on the fluorescent properties of AGEs (Monnier and Cerami., 1981). After the excitation at 370 nm, fluorescence emission wavelength is typically at 440 nm due to the presence of heterocyclic compounds. However, the exact quantitation of AGEs is difficult to achieve due to the lack of proper means to represent the whole diverse AGE family. An alternative method has been suggested for quantitation involving the formation of a chromophore after reacting AGEs with diazonium salts and subsequently measuring absorbance at 490 nm wavelength (Candiano et al., 1986). Recently, the use of immunoassays has been incorporated into many AGE investigations, including research on 2-furoyl-4(5)-(2-furanyl)-1H-imidazole (FFI) (Chang et al., 1985), pyrraline (Miyata and Monnier., 1992), lipoprotein AGEs (Doucet et al., 1995), CML (Reddy et al., 1995), pentosidine
Fig. 4 Schematic representation of pathways leading to AGE formation. The abbreviations given above are represented as: 3-DG (3-deoxyglucosone), CML (N\textsuperscript{ε}-[carboxymethyl]-lysine), CEL (N\textsuperscript{ε}-[carboxyethyl]-lysine), DOLD (deoxyglucosone-lysine dimer), MOLD (methyl glyoxal-lysine dimer), GOLD (glyoxal-lysine dimer).

Source: (Singh et al., 2001)
(Miyata et al., 1996), ribonuclease AGEs (Khalifah et al., 1996), AGE crosslinks (Vasan et al., 1996) and imidazolones (Niwa et al., 1997). However, the antibodies produced may recognize only a limited number of AGEs and leave others undetected. Despite their widespread distribution and their role in pathological consequences, currently there is no universally accepted method to detect AGEs, no internal standards, nor an internationally recognized standard unit of measurement. But recent studies on this area of research will probably lead to some promising results.

**Glycotoxins**

In the process of glycation, AGE peptides are released as degradation products, which partly occur through proteolysis of the matrix component commonly named ‘glycotoxins’. Glycotoxins are very reactive on entering blood circulation. In case they have not been eliminated through the kidney, recirculating AGE peptides can generate new AGE products that react with other plasma or tissue components. At this stage, glycation becomes an autonomic process, which significantly accelerates the process of the secondary complications in various diseases (Turk et al., 2001).

**AGE inhibitors**

AGEs may serve as contributors to the pathogenesis of some chronic disorders (Lee and Cerami., 1990). However, AGEs are recognized as foreign compounds by the human body and can slowly be removed by phagocytosis (Radoff et al., 1988). Pharmaceutical inhibitors of AGE formation which have been investigated include aminoguanidine (Brownlee et al., 1986), ibuprofen, glutathione, aspirin (Ajiboye and Harding., 1989), diclofenac (Van Boekel et al., 1991) and Tenilsetam (Munch et al., 1994). Of these, aminoguanidine has received most of the attention for its inhibitory effect of AGE formation, such as reducing AGE accumulation in collagen and in kidney glomerular basement membrane; postponing the beginning of diabetes-related vasodilatory abnormalities, retinal vascular lesions and nephropathy; and lessening neuropathology in streptozotocin-induced diabetic rats (Ellis and Good., 1991; Itakura et al., 1991; Oxlund and Andreassen., 1992; Yagihashi et al., 1992; Huijiberts et al., 1993; Hammes et al., 1994). Aminoguanidine reacts with the AGE dicarbonyl group, which then cannot crosslink with other macromolecules (Chen and Cerami., 1993).
Free radical generation

It is believed that life originated as a result of free radical reactions. Selected free radical reactions play major metabolic roles, causing repeated mutation and death, thereby assuring evolution (Harman., 2001). Oxygen free radicals are believed to be generated by a number of processes in vivo, including the ‘respiratory burst’ of phagocytic cells, metal catalysed substrate autoxidations, mitochondrial electron transfer and the reduction of hydroperoxides (Halliwell and Gutteridge., 1984). One important source of free radicals is AGEs resulting from non-enzymatic glycation and oxidation of proteins and lipids (Thomas et al., 2005). AGEs generated oxygen free radicals may potentiate the development of atherosclerosis, various autoimmune diseases such as rheumatoid arthritis and diabetes mellitus, and might encourage the aggregation of human platelets through oxidative stress (Hangaishi et al., 1998).

Glycoxidation

Post-translational non-enzymatic protein modifications can occur inside and outside the cell (Fluckiger et al., 1984) and they considered as possible mechanisms of pathogenesis of some chronic diseases. Among the non-enzymatic processes, oxidative stress (Harman., 1992) and non enzymatic glycation (Monnier and Gerontol., 1990) have aroused a particular interest: they are likely to be the basis of the molecular changes leading to cell dysfunction and tissue alterations typical of rheumatoid arthritis, aging and diabetes.

Glucose exists in equilibrium with their enediol, which can undergo autoxidation to form an enediol radical. This radical produces molecular oxygen to generate the superoxide radical and becomes oxidized to a dicarbonyl ketoaldehyde that reacts with protein amino groups forming a ketoamine. Ketoamine are similar to, although more reactive than, Amadori products and participate in AGE formation (Ahmed., 2005). Glycation is a major source of reactive oxygen species (ROS) i.e. generated by oxidative pathways of glycation (Rahbar and Figarola., 2003). Some in vitro studies have been carried out in rat collagen in which it has been find out that glycation and oxidation are closely linked processes (Traverso et al., 1997) and a new term has been coined called ‘glycoxidation’. The two best characterized AGEs that have been formed by glycoxidation are CML and pentosidine. Evidence from this
comes from in vitro experiments in which anti-oxidants resulted in a reduction in CML and pentosidine formation. These glyoxidation products accumulate with time and result in irreversible structural alterations of proteins.

**Protein carbonyl**

Carbonyl groups may be introduced into proteins by reactions with reactive carbonyl derivatives (ketoamines, ketoaldehydes, deoxyosones) generated as a consequence of the reaction of reducing sugars or their oxidation products with amino groups of proteins (Kristal and Yu., 1992; Monnier et al., 1995; Baynes., 1996). Accumulation of protein carbonyls is taken as a biomarker of oxidative protein damage in rheumatoid arthritis, atherosclerosis, hypertension, aging, chronic renal failure, Alzheimer’s disease, sclerosis, cancer, diabetic and uraemic complications (Dalle-Dome et al., 2003; Yilmaz et al., 2003; Ceriello., 2008; Ghafourifar et al., 2008).

Many in vitro studies have shown oxidative damage to proteins results in the formation of carbonyl groups (Oliver et al., 1987; Starke et al., 1987; Starke-Reed and Oliver., 1989; Takahashi and Goto., 1990; Zhou and Gafni., 1991; Hu., 1994). The structure and activity of oxidized proteins change profoundly when compared with their native forms. Therefore, evaluation of the carbonyl group content in plasma proteins is an acknowledged marker for the intensity of oxidation.

**Rheumatoid arthritis**

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that affects ~ 1% of the adult population worldwide with females affected more frequently than males. It is mainly characterized by the progressive and irreversible destruction of joints (Harris et al., 1970). It can also produce diffuse inflammation in the lungs, pericardium, pleura, and the sclera of the eye, and also nodular lesions, most common in subcutaneous tissue under the skin. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility. Even though it is known that persistent activation of the immune system in RA leads to autoimmunity, the trigger activating the immune response remains unclear (Goronzy and Weyand., 2005). The exact pathogenesis is still unknown and the treatment is noncurative. The
presence of rheumatoid factor (autoantibodies against the Fc portion of IgG) (Weyand et al., 1992; Wagner et al., 1997; Ligier et al., 1998) and circulating IgM anti-IgG advanced glycation end products antibodies (anti-AGE-IgG antibodies) (Eberhardt et al., 1990; Ligier et al., 1998; Scofield et al., 1998; Lucey et al., 2000) have served as long-term outcome predictors of RA. The most definite genetic association with RA is with human leucocyte antigen alleles (HLA) of the major histocompatibility complex (MHC). The HLA-DR4 allele which is found at the HLA-DRB1 locus is associated with the development and severity of RA (Weyand et al., 1992; Wagner et al., 1997). Studies have also revealed variation of the genes for various proteins known to drive inflammatory process in RA.

The name is based on the term “rheumatic fever”, an illness which includes joint pain and is derived from the Greek word ‘rheumatos’ (flowing). The suffix ‘–oid’ (resembling) gives the translation as joint inflammation that resembles rheumatic fever. The first recognized description of RA was made in 1800 by Dr Augustin Jacob Landre-Beauvais (1772-1840) of Paris (Landre-Beauvais, 2001). The term “rheumatoid arthritis” was coined in 1859 by British rheumatologist Dr Alfred Baring Garrod (Storey., 2001). It is diagnosed chiefly on symptoms and signs, but also with blood tests such as the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factor (RF) positivity; HLA-DRB1 genotypes, elevated serum AGE-IgG level and radiographic damage. CRP is synthesized by hepatocytes in response to proinflammatory cytokines in particular IL-6. It has been shown to be of great value as an inflammatory marker in RA and has been suggested to mediate part of the complement activation in RA (Molenaar et al., 2001). Normally there is no CRP in blood serum. Although a result of 1 mg/dl is usually considered high for CRP, but most infections and inflammations result in CRP levels above 10 mg/dl. Both CRP and ESR give information about the extent of inflammation in RA. X-rays of the hands and feet in RA patients may not show any changes in the early stages of the disease, but more advanced cases demonstrate erosions and bone resorption.

RA is accompanied by the occurrence of many autoantibodies in the serum of the patients. The possible pathogenic nature of autoantibodies in RA is still controversial. With more sophisticated types of therapy becoming available, it is becoming more important to diagnose RA at an early stage of the disease. So that
earlier treatment can be applied and major damage of joints can be prevented (Smolen and Steiner., 1998; Goldbach-Mansky and Lipsky., 2003).

**Diagnostic criteria of RA**

The American College of Rheumatology (ACR) has defined (1987) the following criteria for the classification of RA (Arnett et al., 1988):

(i) Morning stiffness of >1 hour most mornings for at least 6 weeks.
(ii) Arthritis and soft-tissue swelling of >3 of 14 joints/joint groups, present for at least 6 weeks.
(iii) Arthritis of hand joints, present for at least 6 weeks.
(iv) Symmetric arthritis, present for at least 6 weeks.
(v) Subcutaneous nodules in specific places.
(vi) Rheumatoid factor at a level above the 95 percentile.
(vii) Radiological changes suggestive of joint erosion.

**Autoantibody production in RA**

The most prominently studied aspect in RA is the production of antibodies against self-antigens (such as the Fc region of IgG and AGE-IgG). Discovery of serum autoantibodies was one of the earliest indications of a role for B cells in RA. Recent studies have shown that early diagnosis and treatment of RA leads to better outcomes (Lard et al., 2001; Moreland and Bridges Jr., 2001), so specific and sensitive serologic tests for early detection of RA are highly desirable. Many autoantibodies have been described in RA (Table 4). Some autoantibodies seem to be relatively specific for RA while other autoantibodies are present in other autoimmune or inflammatory diseases such as rheumatoid factor (RF), anti-RA33 antibodies, anticalpastatin antibodies, anticollagen type 2 antibodies etc.

**Rheumatoid factor**

It is defined as an antibody that directly binds to the Fc portion of normal human IgG. It was described in 1940 by Waaler (Waaler., 1940). RFs are found in multiple immunoglobulin isotypes (IgE, IgM, IgA and IgG), although IgM is the RF
<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody</th>
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<tr>
<td><strong>Immunoglobulin antigens</strong></td>
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<tr>
<td>Fc portion of IgG</td>
<td>Rheumatoid factor</td>
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<tr>
<td>AGE-IgG</td>
<td>Anti-AGE-IgG</td>
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<tr>
<td><strong>Citrulline antigens</strong></td>
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<td>Citrullinated peptides on multiple proteins</td>
<td>Anti-cyclic citrullinated peptides</td>
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<td>(Pro)filaggrin</td>
<td>Antiperinuclear factor</td>
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<td>(Pro)filaggrin</td>
<td>Antikeratin</td>
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<td>Filaggrin</td>
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<td>Citrullinated vimentin</td>
<td>Anti-Sa</td>
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<td><strong>Cartilage antigens</strong></td>
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<td>Type II collagen</td>
<td>Anti-collagen</td>
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<td>Human cartilage glycoprotein-39</td>
<td>Anti-HC gp-39</td>
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<td>Aggrecan</td>
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<tr>
<td><strong>Enzymes of the glycolytic pathway</strong></td>
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<tr>
<td>Glucose-6 phosphate isomerase</td>
<td>Anti-GPI</td>
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<td>Enolase</td>
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<td>Creatine kinase</td>
<td>Anti-creatine kinase</td>
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<td><strong>Other antigens</strong></td>
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<td>A2 protein of heterogenous nuclear ribonucleoprotein</td>
<td>Anti-RA33</td>
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<td>Calpastatin</td>
<td>Anti-calpastatin</td>
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<tr>
<td>Heavy chain binding protein (BiP)</td>
<td>Anti-p68</td>
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<tr>
<td>Fibronectin</td>
<td>Anti-fibronectin</td>
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<tr>
<td>Actin, myosin</td>
<td>Anti-cytoskeletal antigens</td>
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<tr>
<td>Pituitary gland-specific factor 1a</td>
<td>Anti-pituitary antigens</td>
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</tbody>
</table>

**Source:** (Bridges Jr., 2004)
measured in clinical assays. Since the presence of RF is one of American College of Rheumatology criteria for diagnosis of RA, the assay is one of the most commonly performed assays in patients with rheumatological illnesses. RF is most clinically useful when there is a moderate pretest likelihood of RA; it is not useful as a screening test for RA in the general population (Shmerling and Delbanco., 1991). RF-positive RA patients had more severe disease, both functionally and radiographically, than RF-negative patients. They also have more extra-articular involvement (including subcutaneous nodules, vasculitis, leg ulcers, and neuropathy) than RF-negative RA patients (Bridges Jr and Davidson., 2005).

Anti-AGE-IgG antibodies

While haemoglobin (glycated form HbA1c), albumin and collagen are common targets of AGE modification in diabetes (Bucala and Cerami., 1992), glycated IgG (AGE-IgG) is associated with inflammation in the context of rheumatoid arthritis (RA). Interestingly, a subset of patients with RA has novel autoantibodies directed against AGE-IgG (i.e. anti-AGE-IgG antibodies) (Ligier et al., 1998; Newkirk et al., 1998; Lucey et al., 2000). Furthermore, elevated levels of glycated γ heavy chains in RA patients, positive for anti-AGE-IgG antibodies, have been observed. However, neither the levels of glycated μ heavy chain nor glycated albumin are increased in the presence of anti-AGE-IgG antibodies, which is compatible with the observation that the autoantibodies are specific for AGE-IgG.

Previous studies have indicated not only that AGE-IgG could be detected in patients with RA, but also that approximately 30-40% of RF-positive patients mounted an immune response against AGE-IgG (Ligier et al., 1998; Newkirk et al., 1998; Tai and Newkirk., 2000). One possible explanation for the origins of these antibodies and the link to RF is that RF-positive B-cells could act as antigen-presenting cells for the damaged IgG and thus stimulate the anti-AGE-IgG response by other antigen-selected B-cells that express a surface immunoglobulin specific for the AGE-IgG. In a previous study of RA patients with longstanding disease (Lucey et al., 2000), the anti-AGE-IgG antibodies were found to correlate significantly with measures of disease activity. It is thus of interest to determine whether the anti-AGE-IgG response is only a feature of longstanding inflammation and RF-positive status, or whether such antibodies could be detected in patients with recent onset of disease
and can AGE-IgG serve as new potential RA marker for evaluating disease status and activity of RA and guide the instillation of more aggressive therapy.

**Anti- cyclic citrullinated peptide (CCP) antibodies**

Citrullinated proteins are present in RA synovium (Baeten *et al.*, 2001). Anti-CCP antibodies are produced locally at the site of inflammation in RA (Reparon-Schuijt *et al.*, 2001). Anti-CCP antibodies and RF are found in the sera of RA patients prior to the onset of RA symptoms. The combination of anti-CCP antibodies and presence of the HLA-DRB1 shared epitope is more predictive of future development of RA than either factor alone (Berglin *et al.*, 2004). The presence of anti-CCP antibodies in early RA is also a good independent predictor for radiographic joint damage and progression.

**Antibodies to antigens found in normal cartilage**

Many antibodies directed at cartilage constituents have been described in RA, such as anti-human cartilage gp-39 (a glycoprotein expressed by articular chondrocytes and synoviocytes), anti-aggrecan and anti-type II collagen (Anti-CII) (Bridges Jr., 2004). The early presence of these antibodies, in conjugation with HLA-DRB1 shared epitope, are associated with rapidly progressive RA (Johansen *et al.*, 1993).

**Antibodies to enzymes of the glycolytic pathway**

Anti-GPI (glucose-6 phosphate isomerase) antibodies have been reported to be more frequent in individuals with extra-articular manifestations of RA (Van Gaalen *et al.*, 2004). Antibodies to other glycolytic enzymes, such as creatine kinase (Schubert *et al.*, 2002) and alpha-enolase have been reported in RA. One study found that anti-alpha-enolase antibodies predicted radiologic progression of RA, and reported specificity for RA of ~97% (Saulot *et al.*, 2002).

**Anti-RA 33 antibodies**

The RA33 antigen is the A2 protein of the heterogeneous nuclear ribonucleoprotein complex. Anti-RA 33 antibodies were described in 1989 and had
been suggested to be a marker for early arthritis (Hassfeld et al., 1989; Hassfeld et al., 1993).

**Anticalpastatin antibodies**

Calpains are calcium-ion-dependent neutral cystein proteinases. Extracellular calpains were found to be increased in the inflamed synovia (Yamamoto et al., 1992). Calpastatin is the natural inhibitor of calpains. Antibodies to calpastatin can be found in 45% of the RA sera. (Despres et al., 1995; Lackner et al., 1998).

**Oxidative stress in RA**

It is well known that oxidative stress is a typical finding in rheumatic joints (Edmonds et al., 1995). Oxygen metabolism has an important role in the pathogenesis of RA. Detection of oxidative stress and low antioxidant concentrations in the plasma of RA patients suggest the involvement of free radicals in inflammatory processes (Frisbie, 2006). Free radicals/ROS produced in the course of cellular oxidative phosphorylation and repetitive cycles of hypoxia and reoxygenation, along with oxidants produced by phagocytic cells such as macrophages and neutrophils, lead to chronic oxidative stress in the RA synovial microenvironment and the other tissues (Hagfors et al., 2003). Increased oxidative stress in synovial tissue and synovial fluid may be associated with increased disease activity, tissue damage and bone erosions in RA. It has been shown that, especially in RA, monocytes produce 2.7 times more oxygen radicals than controls (Ostrakhovitch and Afanas’ev., 2001). Assessment of such biochemical markers as protein carbonyl content is considered as one of the best possible ways to monitor inflammation in RA (Shacter., 2000).

It has been well documented that free radicals have been reported to bring about AGE formation (Baynes., 1991). Oxidative stress accelerates the production of AGE products. According to previous studies, oxygen free radicals have been identified in the synovial fluid of 90% of patients with RA (Lunec et al., 1981). It has been reported that IgG is quite vulnerable to ROS (Griffiths and Lunec., 1989; Swaak et al., 1989; Kleinveld et al., 1991; Maninger et al., 1996; Uesugi et al., 2000; Rasheed., 2008). Many studies showed the presence of elevated levels of oxidized IgG in patients with RA (Swaak et al., 1989; Griffiths and Lunec., 1996). Hence, IgG
is continuously exposed to oxidative stress during inflammation in RA. This oxidative stress in RA patients has been shown to cause IgG to be modified by AGEs (Newkirk et al., 2003). Such glycation of IgG has been correlated with oxidative and inflammatory processes and not with glucose levels i.e. there is no role of hyperglycemia in such cases and glycation is carried out at physiological glucose concentration under oxidative stress.

The well characterized AGE, Nε-carboxymethyllysine (CML), represents a general marker of oxidative stress and tissue damage through protein alteration. CML was detected in the samples of synovial tissue from patients with RA. The accumulation of CML in RA patients’ synovial tissue might be the result of oxidative stress during local and systemic inflammation (Ames et al., 1999).

The antioxidant defense system has been shown to be compromised in RA patients. Studies have shown elevated blood malondialdehyde (MDA) levels in RA patients and significantly lower levels of blood concentrations of total thiols, glutathione, and vitamin C compared to controls (Mantle et al., 1999; Jaswal et al., 2003; Nagler et al., 2003). These studies have found a shift in the oxidant/antioxidant balance in favor of lipid peroxidation, which could lead to the tissue damage observed in the disease. A statistically significant increase in the concentrations of antioxidants, along with a decrease in the concentrations of MDA was found after treatment of the disease (Jaswal et al., 2003). These results suggest the importance of therapeutic coadministration of antioxidants along with conventional drugs to such patients.

**Epidemiology and prevalence of RA**

The incidence of RA is in the region of 3 cases per 10,000 population per annum. Onset is uncommon under the age of 15 and from then on the incidence rises with age until the age of 80. The prevalence rate is 1% with women affected three times more often than men. The risk of first developing the disease appears to be greatest for women between 40 and 50 years of age, and for men somewhat later (Alamanos et al., 2006). RA is 4 times more common in smokers than non-smokers. Female sex hormones may play a protective role in RA; for example, the use of the oral contraceptive pill and pregnancy are both associated with a decreased risk (Hannaford et al., 1990; Brennan et al., 1997). However, the postpartum period has
been highlighted as a risk period for the development of RA (Silman et al., 1992; Nelson and Ostensen., 1997). Furthermore, breastfeeding after a first pregnancy poses the greatest risk (Brennan and Silman., 1994). Exposure to infection may act as a trigger for RA, and a number of agents have been implicated (e.g. Epstein–Barr virus, parvovirus and some bacteria such as Proteus and Mycoplasma) (Silman and Pearson., 2002). RA is strongly associated with the inherited tissue type MHC antigen HLA-DR4, hence family history is an important risk factor. First-degree relatives’ prevalence rate is 2-3% and disease concordance in monozygotic twins is approximately 15-20% (Symmons et al., 2002).

Some Native American groups have higher prevalence rates (5-6%) and people from the Caribbean region have lower prevalence rates. In India, the prevalence of RA is 0.75% (Mijiyawa., 1995). In Indonesia and the Philippines, in contrast, RA appears rare (prevalence below 0.4%), in both urban and rural areas. The rarity of RA in rural Africa contrasts with the high prevalence of the disease in Jamaica, where over 2% of the adult population are affected (Mijiyawa., 1995). Studies in populations from Southeast Asia (Dans et al., 1997), including China and Japan (Zeng et al., 1997; Shichikawa et al., 1999) have shown very low occurrences (0.2–0.3%).

**Objectives of the present study**

Glycation of IgG is of special interest as AGE-IgG is associated with inflammation in RA and a subset of RA patients is found to contain novel autoantibodies directed against AGE-IgG. In the present study, human IgG was incubated with physiological concentration of glucose (5 mM) for different time periods. The physico-chemical changes induced by glycation in IgG have been studied by different spectroscopic techniques including, UV-visible, fluorescence, circular dichroism (CD) and fourier transform infrared (FT-IR) spectroscopy, thermal denaturation, high performance liquid chromatography (HPLC) and native and SDS-PAGE. For the detection of early glycation products, nitroblue tetrazolium (NBT) and thiobarbituric acid (TBA) assays were performed. Oxidative damage to the protein upon glycation was evaluated by the estimation of protein carbonyl content and free sulphhydryl group content. Polyclonal antibodies against native and AGE-IgG have
been generated in experimental animals and their antigenicity was assessed by direct binding ELISA. The specificity of induced antibodies has been evaluated by competition ELISA and gel retardation assay. In order to assess the possible role of AGE-IgG in the pathogenesis of RA, sera of RA patients have been investigated for the presence of antibodies against native and AGE-IgG.