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Review article

Where disease pathogenesis meets protein formulation: Renal deposition of immunoglobulin aggregates

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Abstract

Aggregation is one of the important issues encountered during the development of immunoglobulin-based drugs. The aim of the current review is to discuss the causes and consequences of immunoglobulin aggregation as well as the relevance of immunoglobulin aggregation to disease pathogenesis. Extracellular deposition of immunoglobulins, either monoclonal light chains or intact polyclonal antibodies, induces renal failure in various nephropathies. The aggregates can present fibrillar or amorphous structures. In this review, factors known to influence protein aggregation, such as the primary structure of the protein, local environment and glycosylation are assessed, as well as the subsequent altered clearance, fibril formation and toxicity. The role of the protein local environment is emphasized. Even if the local environment causes only minor perturbations in the protein structure, these perturbations might be sufficient to trigger aggregate formation. This fact underlines the importance of choosing appropriate formulations for protein drugs. If the formulation provides a slightly destabilizing environment to the protein, the long-term stability of the drug may be compromised by aggregate formation.

Keywords: Immunoglobulin; Aggregation; Disease; Formulation; Therapeutic proteins; Amyloid

1. Introduction

There are many classes of protein where aggregation is associated with a disease. Over the last decades, much research has focused on proteins such as the amyloid beta protein and alpha synuclein. Less known is the fact that the aggregation of monoclonal or polyclonal immunoglobulins and subsequent renal damage take place in fatal diseases such as primary (AL) amyloidosis, light-chain deposition disease, heavy-chain deposition disease, light- and heavy-chain deposition disease, cast nephropathy and IgA nephropathy (Berger's disease) [1,2]. Although IgA nephropathy has a higher prevalence in Asian countries, representing 40–50% of all glomerulonephritis in Japan, Singapore and Hong Kong, IgA nephropathy has also become the most common form of glomerulonephritis in other industrialized countries [3,4].

Fig. 1 schematically presents the different diseases caused by immunoglobulin aggregation and Table 1 summarizes the relevant literature for each disease. In AL amyloidosis, monoclonal immunoglobulin light chains are deposited in the form of amyloid fibrils. In the case of light-chain, heavy-chain and light- and heavy-chain deposition diseases (LHCDD), which are different forms of the same pathology, monoclonal light or heavy chains are deposited in the form of amorphous aggregates [5]. These aggregates (fibrillar or amorphous) are found in the glomeruli and eventually cause renal failure. Their presence can also be detected in other tissues; cardiac deposits for instance, present in $>50\%$ cases of AL amyloidosis, are associated with shorter survival [6,7]. In cast nephropathy or 'myeloma kidney', filtered light chains bind to Tamm-Horsfall Protein (THP) in the thick ascending limb of the loop of Henle and form aggregates, obstructing the tubule fluid flow [8,9]. In IgA nephropathy, polyclonal intact IgA and complement component 3 (C3) deposit in the glomeruli, causing a glomerulonephropathy [2].

Immunoglobulins are present in relatively high concentrations in the blood stream, and even though they share most of their structure, their different aggregation patterns lead to

Abbreviations: C3, complement component 3; CDR, complementarity-determining region; COS-1, African green monkey kidney cells; DTT, dithiothreitol; FcαR, Fc receptor for IgA; IgA, immunoglobulin A; IgG, immunoglobulin G; IL, interleukin; LHCDD, light-chain, heavy-chain or light- and heavy-chain deposition disease; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TGF, transforming growth factor; THP, Tamm-Horsfall protein; TNF, tumour necrosis factor.

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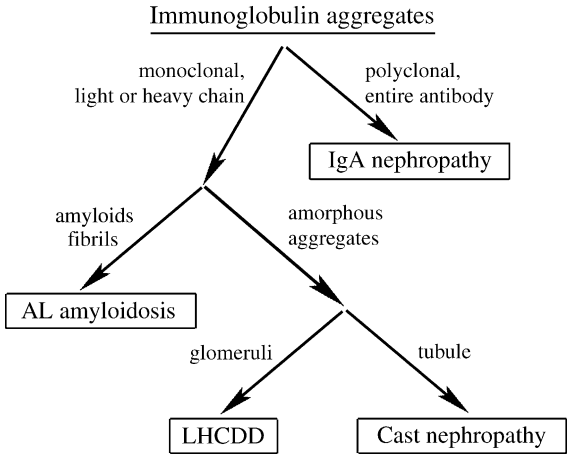

Fig. 1. Schematic description of the different diseases (in boxes) caused by immunoglobulin aggregation. The diseases are classified according to the following parameters: the nature of the protein, the type of aggregates and the localization of the renal damage.

different diseases. The fact that the aggregation of such endogenous proteins can cause a fatal disease highlights the importance of understanding the factors influencing immunoglobulin aggregation.

Immunoglobulins are also used as drugs and currently there are more than a dozen products on the market. Aggregation is one of the issues encountered during formulation that often compromises the stability of protein drugs. Little is known about the fate of the therapeutic immunoglobulins once injected into the human body and minimizing aggregation is one step towards reducing potential toxicity of protein drugs.

In this review, we discuss the potential causes of immunoglobulin aggregation in renal diseases and implications on the rational development of stable pharmaceutical formulations of proteins. Relevant factors influencing protein aggregation, such as the immunoglobulins' primary structures, their local environment and their glycosylation will be presented. The current knowledge regarding the mechanisms governing aggregate formation will be summarized. The toxicity of the aggregates to the kidney will also be described.

2. Causes of immunoglobulin aggregation

2.1. Influence of the primary structure

In AL amyloidosis, LHCDD and cast nephropathy, the immunoglobulin deposits are constituted of monoclonal light chains and light chain fragments. These light chains can either form fibrillar (amyloid) or amorphous aggregates, and present different tropisms. The fact that the immunoglobulins responsible for the disease come from a single clone has suggested that the amino acid sequence of the protein could explain the tendency to aggregate [1].

In order to describe the primary structure elements, which could cause these diseases, two factors were considered: the light chain subtype and the amino acid mutations. Immunoglobulin light chains are either from the κ or from the λ type. Each of these types is divided in subtypes: κ_I to κ_{IV} and λ_I to λ_{VIII} [10]. Serologic studies and sequence analysis of amyloid deposits extracted from more than 50 patients have shown that almost all subtypes can be involved in AL amyloidosis [1]. Another study has demonstrated that the λ_{II} subtype was overexpressed in patients suffering from AL amyloidosis, multiple myeloma or Waldenström's macroglobulinemia [11]. However, only the λ_{VI} subtype has been recognised as particularly amyloidogenic and is found exclusively in patients with AL amyloidosis [11].

As opposed to AL amyloidosis, κ light chains are involved in LHCDD twice as often as λ light chains, but less structural data are available and no particular subtype has been associated with the disease. In cast nephropathy, the tendency of light chains to aggregate seems to be independent of the light chain type [5]. This could be explained by the fact that in cast nephropathy, aggregates are formed through the interaction of light chains with Tamm-Horsfall Protein, and this interaction takes place in the complementarity-determining region (CDR) 3 [12]. As the domain responsible for aggregation is located in one of the hypervariable regions, the light chain subtype is less susceptible to being directly involved in aggregation.

Even though in AL amyloidosis, amyloid material contains fragments of the *constant* region [13–15]; the main constituent of the aggregates is the *variable* region of the light chain. The natural variability of this domain mitigates against the designation of a single amino acid or an amino acid sequence as responsible for aggregation. Furthermore, amyloid fibrils are cross-β-structures and light chains natively contain a considerable amount of β-structure. One situation where single amino acid substitutions could trigger aggregate formation would be by promoting the exposure of hydrophobic residues, destabilization of secondary structures, enhanced sensitivity to proteolysis or an increase in the population of intermediates in the folding–unfolding process. For example, Pro-40 is a highly conserved amino acid in immunoglobulin light chains [16] and a single mutation involving replacement with Leu could be responsible for fibril formation [17]. In this case,

Table 1
Classification of immunoglobulin-related diseases and corresponding literature

Disease	Precursor protein	References
AL amyloidosis	Monoclonal light chain, predominantly of the λ subtype	[1,6,7,11,13–16,18–23,25,28–30,32,35,36,42–45,47–52,56,62,68–70,72,73,76–78,81]
LHCDD[a]	Monoclonal light chain, predominantly of the κ subtype	[1,5,19,25–27,34,35,78]
Cast nephropathy	Monoclonal light chain	[8,9,12,54,70]
IgA nephropathy	Polyclonal entire IgA	[2–4,57–61,63–67,79,80,82–93]

[a] Light-chain, heavy-chain and light- and heavy-chain deposition diseases.