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MOLECULAR STRUCTURE, FUNCTION AND THERAPEUTIC ENTITIES OF IMMUNOGLOBULIN

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ABSTRACT

Immunoglobulins are heterodimeric proteins made up of two heavy (H) and two light (L) chains. They can functionally be divided into variable (V) domains that bind antigens and constant (C) domains that determine effector functions, such as complement activation or Fc receptor binding. The variable domains are produced by a complex series of events in gene rearrangement and can then be subjected to somatic hypermutation after antigen exposure. Each V domain can be divided into three regions of sequence variability, called CDRs, or complementarity determining regions, and four regions called framework regions, or FRs, of relatively constant sequence. The three CDRs of the heavy chain are combined with the three CDRs of the light chain to procedure the antigen binding site. Five major groups of heavy chain C domains exist. The isotypes of IgM, IgG, IgA, IgD, and IgE are defined by each class. To activate altered effector function while preserving antigen specificity, the constant domains of the heavy chain can be switched. The aim of this review is to review current development in our understanding of the structure, function and therapeutic effects of immunoglobulin, as well as the immunological implications of Ig for the management of autoimmune diseases and cancer. For that reason, a literature search on PubMed and Google Scholar was carried out using the specific keywords.

KEY WORDS: *Immunoglobulin, Antibody function; Immunoglobulin gene rearrangement; Class switching; Somatic hypermutation.*

INTRODUCTION.

The ultimate mission of the immune system is to identify and destroy extraneous molecules attacking the host. A foreign substance must respond with fixed or circulating receptors, which initiate the final response, in order to be triggered. Usually, two separates but strictly co-operating systems accomplish this mission. The innate immune system involves the cells of reticuloendothelial system (RES), the complement cascade and the mediators generated and released by these cells through the interaction among the host and the invading organism (Yatim & Lakkis, 2015). The number of receptors present on the surface of cells of the innate immune system is genetically determined and cannot match the enormous variability of microbial antigenic epitopes. In order to face the multitude of agents and/or substances that come into contact with the host, a more flexible mechanism is needed. This second pathway, referred as adaptive immunity, involves immunoglobulins, which are encoded by genes that can undergo somatic recombination and hypermutation due to their ability to deal with

continuously changing antigens (Nicholson, 2016). Immunoglobulins are naturally produced by plasma cells produced from B lymphocytes, which are triggered by trapping antigens on a cell-surface receptor and with CD4+ T lymphocytes stimulation. Generally, antibodies divided into five different classes (IgG, IgA, IgM, IgE, and IgD) (Berlot et al., 2015). it is confirmed that immunoglobulin is Y-shaped proteins composed of two identical heavy chains and two identical light chains (LCs) (Chiu et al., 2019). Currently, the Protein Data Bank has identified and deposited over1,000 antibody Fab or Fab variable (Fv) structures. Many of these antibodies target proteins found in infectious agents, such as viruses or bacteria, or molecules that are secreted by them. It is reported that one Fab antibody fragment may look like another at first glance, but closer analysis reveals that these adaptive immune system workhorses may capitalize on new structural features to customize their binding sites to meet targets of different shapes, sizes, and properties. Over recent decades, immunotherapies, including checkpoint inhibitors, adoptive cell transfer, monoclonal antibodies, and vaccine therapies, have become effective and highly specific therapies to treat cancer by improving the immune system of a patient. These therapies can directly target tumor cells with fewer cytotoxicity and less adverse effects, as well as the tumor microenvironment (Kimiz-Gebologlu et al., 2018).

The aim of this review is to explore latest developments in our understanding of the structure, function and therapeutic uses of immunoglobulin, as well as the immunological aspects of Ig for the treatment of autoimmune diseases and cancer. A literature search on PubMed and Google Scholar was carried out using the keywords. More than 40 PubMed documents and papers have been re-tried using the key words 'Immunoglobulin and antibody. A selection of papers from PubMed were collected from another combination of keywords. Using the combination of keywords 'Immunotherapy, Ig structure and Ig feature, the most recent and most cited papers from Google Scholar database has been collected. The findings have been reviewed for further analysis, duplications have been eliminated and similar papers in English are highlighted. All the articles used were referenced in the list of references. The related knowledge relating to the paper goals was then used in order to produce collective data and make explanations to achieve the goals (Kimiz-Gebologlu et al., 2018).

BASIC. STRUCTURE OF. IMMUNOGLOBULIN

Immunoglobulins are heterodimeric proteins composed of two heavy and two light chains. They can be separated functionally into variable (V) domains that binds antigens and constant (C) domains that identify effector roles such as complement activation or binding to Fc receptors. Naturally, the combination of one light chain (LC) with one heavy chain (HC) links with another identical heterodimer to form the complete immunoglobulin. The heterodimer's HC and LC are linked by disulfide bonds. Disulfide bridges also join the two HCs of the heterotetramer (Figure 1). The two HCs of the heterotetramer are also combined by disulfide bridges (Figure 1). Human LCs can be one of two functionally similar classes, κ or λ. Both LC classes have two domains, a constant light domain (CL) and a variable light domain (VL) (Chiu et al., 2019).

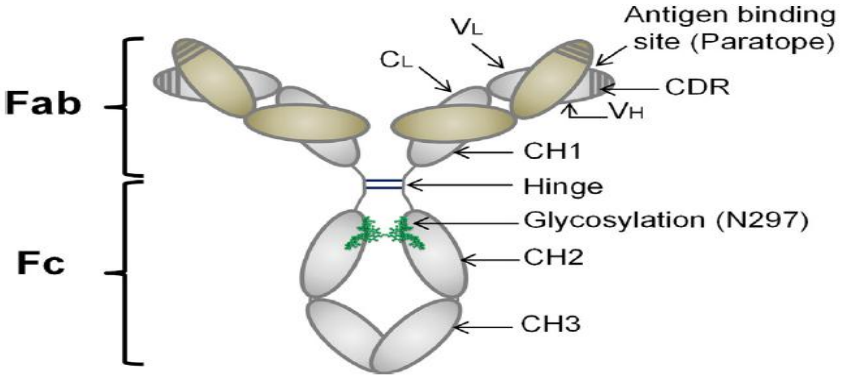

Figure 1: Structure of the antibody: A diagram representing the basic structure of the antibody and the corresponding nomenclature. The light chain (shown in brown) consists of a domain of variables (VL) and constants (CL), while the heavy chain (shown in grey) consists of a domain of variables (VH) and three domains of constants (CL) (CH1, CH2 and CH3). The regions defining complementarity (CDR, seen as striped lines) determine antigen specificity.

In comparison, human antibody heavy chains can be one of five isotypes, IgA, IgD, IgE, IgG, and IgM, each with an independent function in the aquired immune system. IgAs, IgDs, and IgGs have three constant domain (C) and one variable domain (V). while, IgEs and IgMs have four constant domains and one variable domain (Figure 2). The additional J- chain can only be seen in IgM and IgA isotopes, which allows the formation of pentamers and dimers, respectively. The remaining classes are monomeric, which means, having only a pair of HC-LCs. It is stated that IgG isotypes can be subdivided into four subclasses, IgG1, IgG2, IgG3, and IgG4, each with its own biologic function. In addition, IgA can also divided into IgA1 and IgA2 (Chiu et al., 2019).

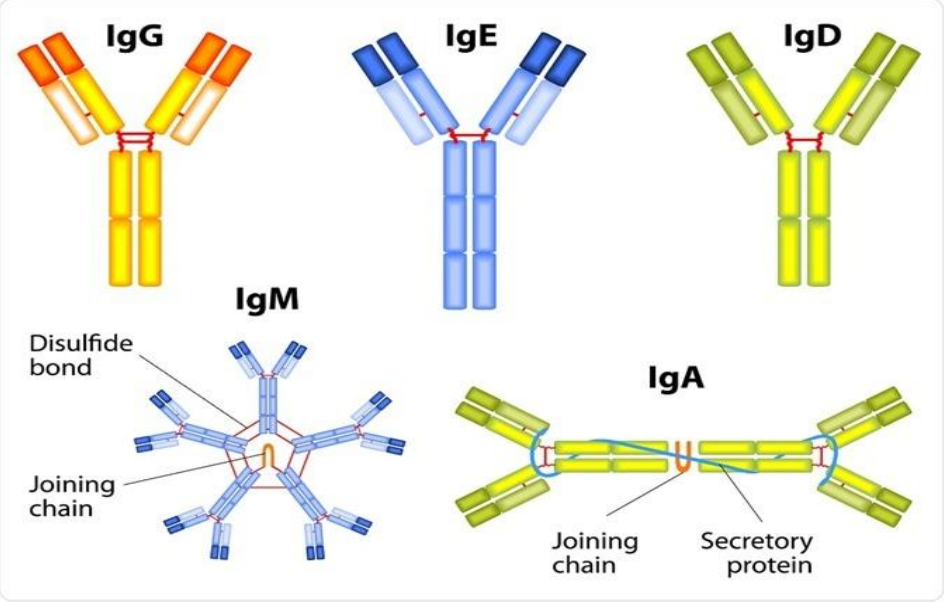

Figure 2: diagram showing structure of Immunoglobulin different classes.

There are three functional components in the intact antibody molecule shown in Figure 3, two Fragment antigen binding domains (Fabs) and the crystallizable fragment (Fc), with the two Fabs attached to the Fc by a hinge region that gives the Fabs a wide degree of flexibility of

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conformation relative to the Fc. For binding to a particular target antigen, each of the Fabs have similar antigen-binding sites. The Fv region of the Fab is make up of a pair of variable domains (V heavy and V light) contributed by the HC and LC. (Chiu et al., 2019).

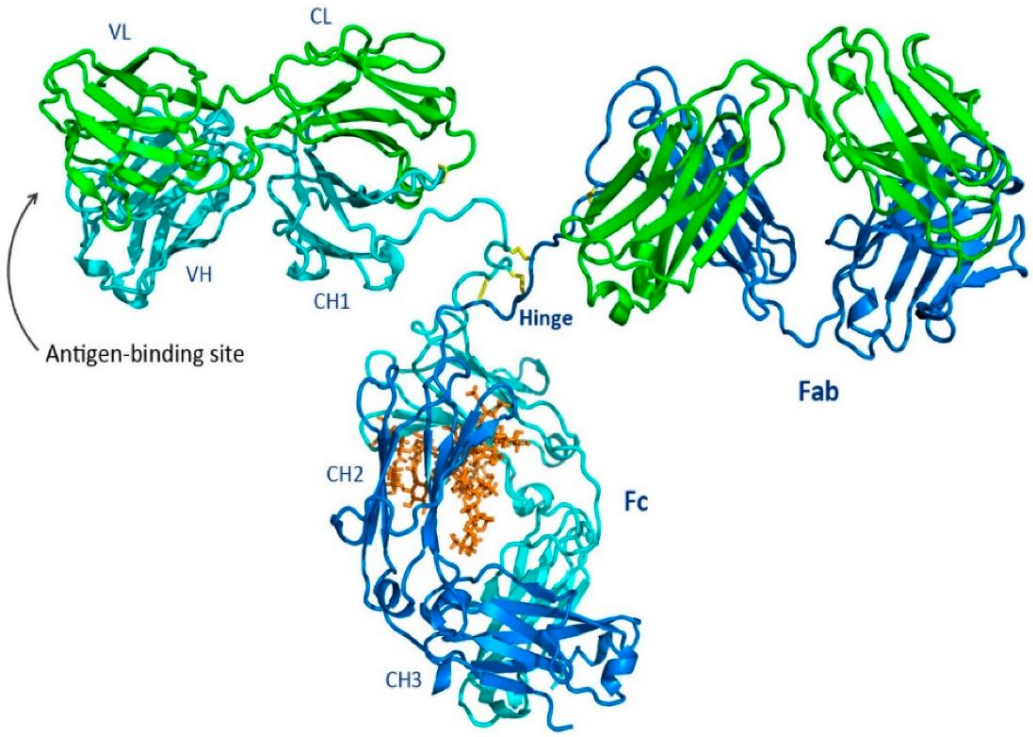

Figure 3. A ribbon representation of whole Immunoglobulin. The light chains are green, the heavy chains are cyan and blue, the orange sticks are glycan, and the yellow sticks are interchain disulfides.

The Immunoglobulin domains

Immunoglobulins (Igs) are belonging to the super-family of eponymous immunoglobulins (IgSF). They consist of two two light (L) and heavy (H) chains, where the L chain can consist of κ or a λ chain. Each chain contains one NH2-terminal variable (V) Immunoglobulin super family (IgSF) domain and one or additional COOH-terminal constant (C) IgSF domains, each of which consists of two sandwiched β pleated sheets pinned together by a disulfide bridge between two conserved cysteine residues (Figure 4). Each constant or variable domain made up of approximately 110–130 amino acids, averaging approximately 12,000–13,000 kDa. Both Immunoglobulin light chains have only one constant domain, while Ig heavy chains contain either 3-4 domains. It is reported that a structure called hinge region located between the first (C_H1) and second (C_H2) domains of H chains holding three C domains. Thus, a standard L chain will weigh about 25 kDa, and a three C domain Cγ H chain with its hinge will weigh about 55 kDa. The amino acids that populate the external surface of the IgSF domain and the loops that bind the β strands are allowed to have considerable variability (Centa et al., 2018).

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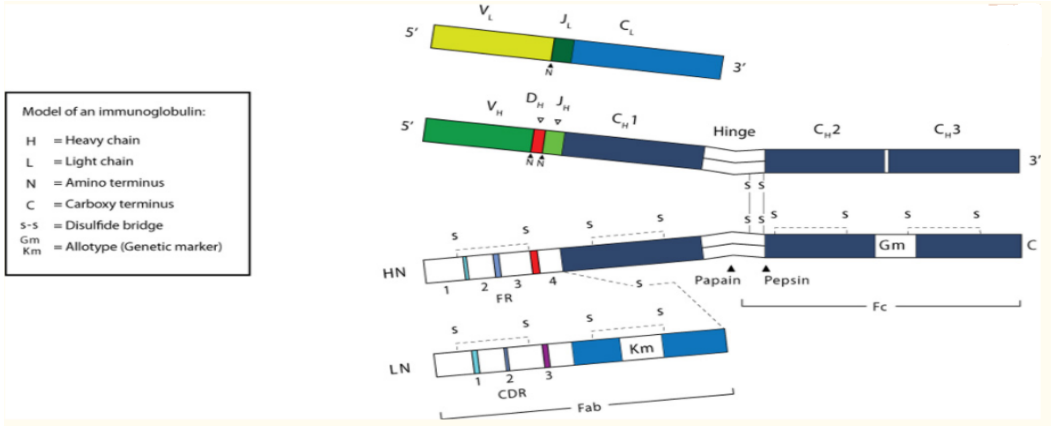

Figure 4: Two-dimensional model of an Ig. The top chains (H and L) deconstruct the antibody at level of nucleotide. The bottom chains deconstruct the protein sequence.

Antigen Recognition and the Fab

The use of enzymes to fragment IgG molecules facilitated early Ig structure studies. IgG is digested into two Fab fragments by the action of Papain, both of which can bind antigen, and a single fragment of Fc. In addition, pepsin divides IgG into an Fc fragment and a single F(ab)2 dimeric that can cross-link and bind antigens. The Fab region comprises one complete (L) chain and the V and C_H1 portion of one (H) chain (Figure 4). It is possible to further divide the Fab into a variable fragment (Fv) composed of the domains VH and VL, and a constant fragment composed of the domains CL and CH1. To recapitulate the monovalent antigen binding features of the original parent antibody, single Fv fragments can be genetically engineered.

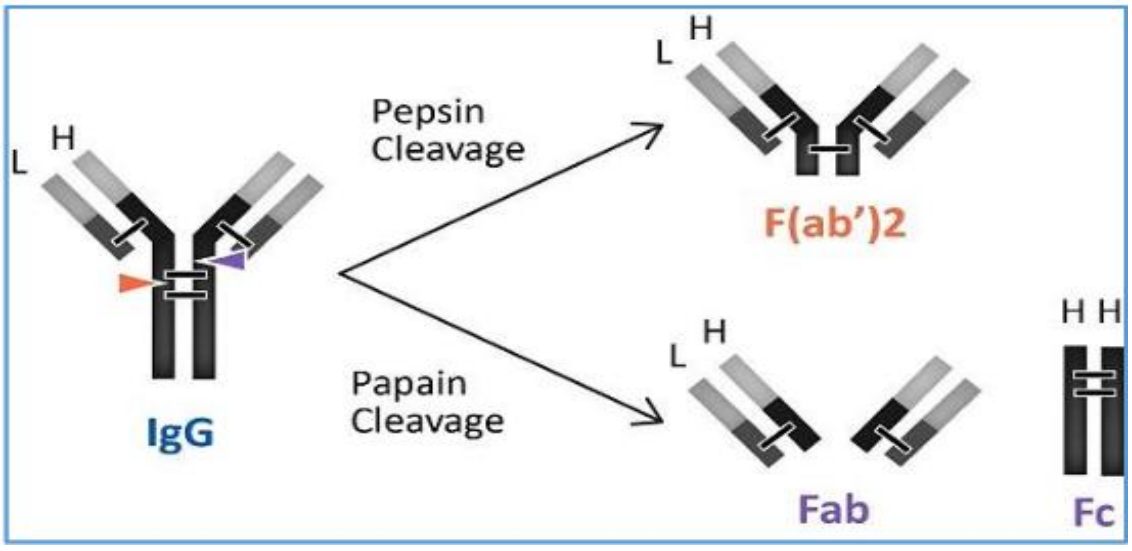

Figure 4: Enzymatic digestion of Immunoglobulin (IgG)

Paratopes, Epitopes, Idiotypes and Isotypes

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Usually, immunoglobulin-antigen interactions take place between the paratope, the Ig site at which the antigen binds, and the epitope, which is the bound antigen site. In vivo, immunoglobulins appear to be formed in soluble form against intact antigens and thus preferentially recognize surface epitopes that can reflect noncontiguous conformational structures in the primary sequence of the antigen. This ability to recognize component fragments of the antigen independently of the rest makes it easier for the B lymphocyte to distinguish between two strongly related antigens, each of which can be regarded as a pool of epitopes. It also helps the same antibody to bind different antigens that share similar or equivalent epitopes, a mechanism referred to as cross-reactivity (Irani et al., 2015).

Immunization of heterologous species with monoclonal antibodies (or a limited collection of immunoglobulins) has allowed both common and individual antigenic determinants of immunoglobulins to be identified. Within V domains, individual determinant(s), called idiotype(s), are included. Common determinants, referred as isotypes, are unique to the constant portion of the antibody and allow immunoglobulins to be grouped into recognized classes, identifying an individual type of C domain for each class. Determinants common to subsets of individuals within a species are called allotypes and describe genetic polymorphisms resulting from gene alleles that differ among other members of that species (Kapingidza et al., 2020).

IMMUNOGLOBULIN GENE REARRANGEMENT AND ORGANIZATION

A separate multigene family encodes Ig heavy and light chains, and the individual V and C domains are each encoded by separate elements: V(D)J gene segments for the V domain and individual exons for the C domains. The primary sequence of the V domain is divided functionally into three hypervariable intervals, named complementarity determining region (CDRs), situated between four stable sequence regions called frameworks (FRs) (Kapingidza et al., 2020).

Immunoglobulin Rearrangement

Usually, each V gene segment contains its own promoter, a leading exon, an interfering intron, an exon encoding the first three framework regions (FR 1, FR 2, and FR 3), the entire CDRs 1 and 2, the amino terminal part of CDR 3, and a recombination signal sequence (RSS). Each J (joining) gene segment starts with its own recombination signal, the carboxy terminal part of CDR 3, and the entire FR 4 (Scheijen et al., 2019). The formation of a V domain is driven by the recombination signal sequences (RSS) that flank the gene segments that are rearranged. Each RSS contains a strongly conserved seven base pair or heptamer sequence (e.g., CACAGTG) separated by either a 12- or 23-base-pair spacer from a less well-conserved nine base pair or nonamer sequence (e.g., ACAAAACCC). These spacers place, on the same side of the DNA molecule, the heptamer and nonamer sequences, separated by either one or two turns of the DNA helix. A recombination signal sequence of one turn (12 base pair spacer) would preferentially recognize a sequence of two turns (23 base pair spacer), thus preventing unnecessary rearrangement of V-V or J-J (Yu & Lieber, 2019). Recombination activating genes 1 and 2 (RAG-1 and RAG-2), which are almost exclusively expressed in developing lymphocytes, are needed to trigger the V(D)J recombination reaction. A DNA double-strand break (DSB) between the terminus of the rearranging gene portion and its

neighboring recombination signal sequence is introduced by RAG-1 and RAG-2. Ubiquitously expressed components of a DNA repair process, known as nonhomologous end-joining (NHEJ), are then repaired by these splits, which are common to all body cells. Thus, although RAG mutations affect only lymphocytes, NHEJ protein loss or alteration-of-function mutations in all cells of the body are susceptible to DNA damage. The NHEJ method produces reliable links between the RSS ends, and imprecise links between the coding ends. Terminal deoxynucleotidyl transfers (TdT) expressed only in lymphocytes may add non-germline encoded nucleotides (N nucleotides) variably to the coding ends of the recombination product.

Class Switch Recombination

Nine functional CH genes are located downstream of the VDJ loci. A series of exons consist of these constant genes, each encoding a different domain, hinge, or terminus. To produce two different types of carboxy termini, all CH genes can undergo alternative splicing: either a membrane terminus that anchors immunoglobulin on the surface of the B lymphocyte or a secreted terminus that occurs in the soluble form of immunoglobulin. With the exception of CH1δ, both an untranslatable exon (an I exon) and a region of repeated DNA called the switch (S) are followed by each CH1 constant region. Initiating transcription and thereby activating the gene by cocktails of cytokine signals transmitted by T cells or other extracellular factors activate the I exon variably. This allows the B cell to change the receptor and the effector ends of the antibody molecule to satisfy a particular need (Yu & Lieber, 2019).

Somatic Hypermutation

A final immunoglobulin diversity pathway is only involved after antigen exposure. With T cell aid, at a rate of up to 10^3 changes per base pair per cell cycle, the variable domain genes of germinal center lymphocytes undergo somatic hypermutation (SHM). SHM is associated with locus transcription and two different mechanisms are involved in humans: the first mechanism targets RGYW (purine/G/pyrimidine/A) motif hot spots mutation (21) and the second mechanism incorporates an error-prone DNA synthesis that can lead to a mismatch of nucleotides between the original template and the mutated DNA strand. To introduce additional somatic diversity, other species use gene transfer between functional and non-functional V sequences. In response to repeated immunization or antigen exposure, SHM enables affinity maturation of the antibody repertoire.

HEAVY CHAIN CONSTANT DOMAIN STRUCTURE AND FUNCTION

In general, the H chain's C domain determines effector function, while the antibody's paired V domains confer antigenic specificity. The constant domain of the H chain is usually specified as CH1-CH2-CH3 (IgG, IgA, IgD) with IgM and IgE as an additional domain (CH4). The CH1 domain is located within the F(ab) region, as mentioned above, while the Fc fragment is comprised of the remaining CH domains (CH2-CH3 or CH2-CH4). This fragment of the Fc describes the immunoglobulin isotype and subclass. Each CH region folds into a fairly constant structure consisting of a three-strand-four strand beta sheet held together by an intrachain disulfide bond, despite amino acid variations between the isotypes and subclasses. Via binding to the Fc receptor on effector cells or triggering additional immune mediators

such as complement; the Fc fragment mediates effector activity. For this cause, differences in the region of Fc may have a major influence on the finale result of the interaction of an antibody-antigen. The Fc region may also affect the affinity or kinetics of the Fv region's binding of the antibody and thus influence the identification or binding of antigen (Lepore et al., 2017).

THERAPEUTIC ENTITIES OF IMMUNOGLOBULINS

Bispecific Antibodies.

Bispecific antibodies form a central component of the next phase of antibody therapy, with the emerging biotherapeutic market dominated by antibody molecules. Two different antigens, such as binding tumor cell receptors concurrently and recruiting cytotoxic immune cells, can be attacked at the same time by Bispecific antibodies. In the bispecific antibody sector, structural diversity has been rapidly increasing, producing a plethora of novel bispecific antibody scaffolds, which provide great functional variety. The single-chain variable fragment (scFv)-based (no Fc fragment) antibody and the full-length IgG-like asymmetric antibody are two common formats of bispecific antibodies on the market. Apart from traditional monoclonal antibodies, significant development problems have hindered their broader clinical application and acceptance with regard to the amount, consistency and stability of bispecific antibodies (Wang et al., 2019).

Single chain variable fragment (scFv) antibodies

Single-chain variable fragments (scFvs) are minimal structures of a functional antibody created by a flexible polypeptide linker by fusing variable domains of the IgG heavy chain (VH) and light chain (VL) (Brinkmann & Kontermann, 2017). The molecules of ScFv have a 25 kDa molecular weight, with a single antigen-binding site consisting of components from each arm of the antibody (Wang et al., 2019). The types of antibody fragment, the linker type, and production capacity are several important considerations in the development of scFv antibodies. More recently, the chimeric antigen receptor (CAR) T-cell approach for adoptive cell transfer immunotherapy is another promising field for using scFv technology (Brinkmann & Kontermann, 2017). Three key bispecific antibody fragment formats are currently available: bispecific T-cell engager (BiTE), dual-affinity re-targeting proteins (DARTs) and Tandem diabodies (TandAbs) (Wang et al., 2019).

In cancer immunotherapy, BiTE molecules have been widely applied to re-target T-cells to tumor cells or tumor-associated cells in the microenvironment of the tumor. They use scFv fragments from two separate monoclonal antibodies linked by a peptide linker, allowing them, when assembled, to maintain the binding activity of each antibody. The short flexible linker connecting the two scFvs allows the two arms to rotate freely, which is necessary for flexible interaction with targeted receptors on two opposing cell membranes (cytotoxic T-cell and tumor cell) and subsequent T-cell activation induction. Blinatumomab, which has been approved by the FDA for the treatment of B-cell precursor acute lymphoblastic leukemia (ALL), is one of the most effective BiTE medicines. Blinatumomab consists of anti-CD 19 scFv in the VL-VH orientation linked to anti-CD3 scFv in the VH-VL orientation through a short glycine/serine (GGGGS) linker (Huehls et al., 2015).

Researchers created a possible solution, dual-affinity re-targeting proteins, partly in response to these problems (DARTs). A DART consists of two fragments of Fv, with two separate sites

of antigen binding formed when two fragments of Fv heterodimerize. Specifically, Fv1 consists of antibody A's VH and antibody B's VL, while Fv2 is made of antibody B's VH and antibody A's VL. This combination enables DART to imitate natural interactions within an IgG molecule, unlike BiTE antibodies bound by a polypeptide linker (Wang et al., 2019). By forming a C-terminal disulfide bridge, adding another cysteine residue to the end of each heavy-chain enhances stability. DART molecules are capable of retaining power for both in vitro and in vivo administration compared to a BiTE, but can be produced at a lower aggregation rate scale. A latest assessment by Moore et al. of CD19xCD3 DART and BiTE molecules' in vitro ability to destroy B-cell lymphoma showed that DART molecules consistently outperformed BiTE molecules (Walseng et al., 2016).

In contrast to natural antibodies, the small size of scFvs leads to a high renal clearance rate. To preserve the avidity of a natural bivalent antibody, these tetravalent bispecific antibodies have two binding sites for each antigen (Reusch et al., 2016). In addition, the molecular weight of TandAbs (approximately 105 kDa) exceeds the first-pass renal clearance threshold, providing a longer half-life compared to smaller antibody constructs (Compte et al., 2014; Reusch et al., 2016). Furthermore, two TandAb format are in clinical phase trials—AFM13 for Natural Killer cell recruitment and AFM11 for T-lymphocyte recruitment (Brinkmann & Kontermann, 2017; Wang et al., 2019).

Full-Size IgG-like Bispecific Antibody

Although IgG-like asymmetric bispecific antibodies have several properties that are analogous to monoclonal antibodies (mAbs), they are bioengineered molecules that have not been produced by typical B-lymphocyte. As a consequence, these variations lead to major challenges in development. Ensuring the proper assembly of antibody fragments is one of the main challenges for the development of asymmetric IgG-like bispecific antibodies, which is a prerequisite for the large-scale production of bispecific antibodies. 16 combinations (10 different molecular configurations) result in a random assembly of four distinctive polypeptide chains (two distinct heavy and two distinct light chains), of which only two represent the desirable asymmetric heterodimeric bispecific antibody (12.5 % of the statistical probability) (Krah et al., 2018). The residual pairings result in monospecific or non-functional molecules (Brinkmann & Kontermann, 2017). Therefore, by optimizing the correct assembly of bispecific antibodies, not only the efficiency, but also the number of bispecific antibodies derived from E coli and mammalian cells can be greatly improved. In order to generate the desired IgG-like bispecific antibody, there are primarily two problems that must be solved: the heterodimerization of two separate heavy chains and the discrimination between the two interactions between the light-chain/heavy-chain.

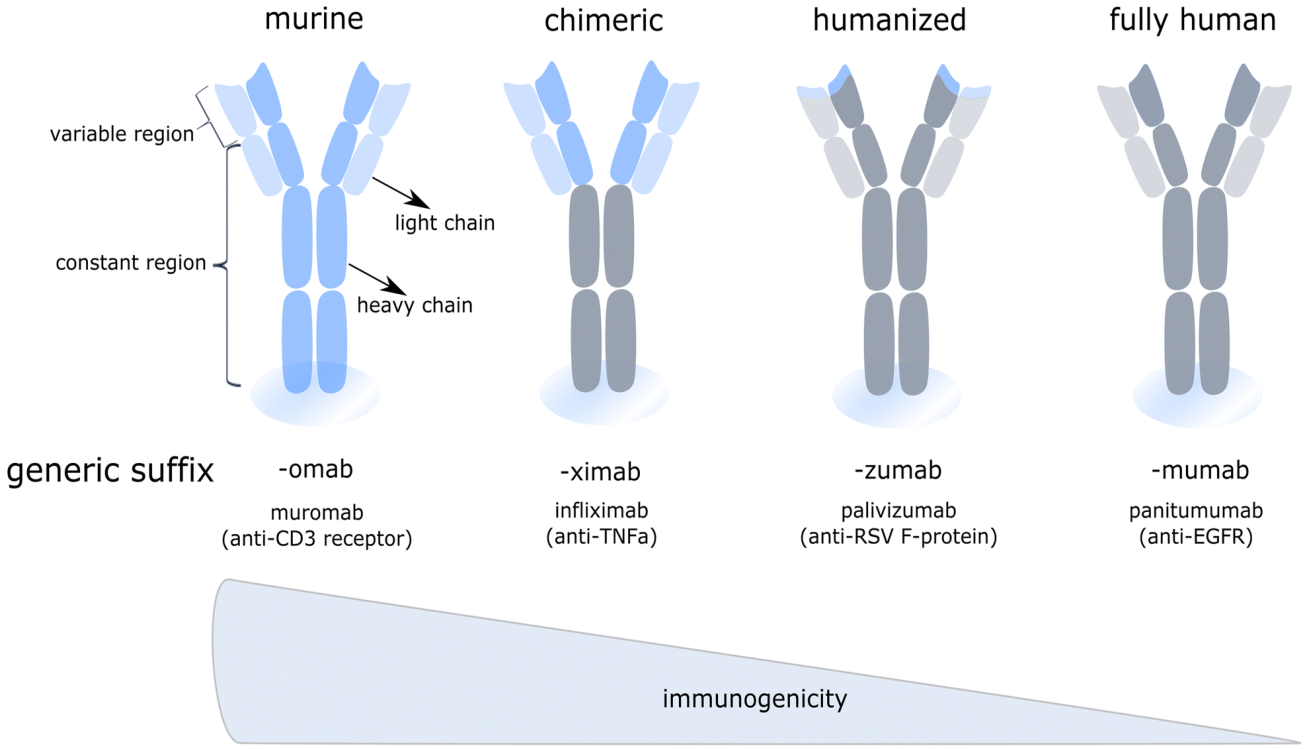
Monoclonal antibodies

An antibody produced against a single antigen epitope is called monoclonal antibody (mAb) which is produced by a single plasma cell type, while polyclonal antibodies bind to multiple antigen epitopes or multiple antigens and are usually produced by multiple plasma cells (Kimiz-Gebologlu et al., 2018; Wang et al., 2019). For over 30 targets and diseases, most notably cancers, monoclonal antibody therapeutics have been approved. The new backbone of the pharmaceutical industry, which historically relied on small molecules, is the use of monoclonal antibodies (Shepard et al., 2017). The first monoclonal antibodies were produced by Köhler and Milstein in 1976 (Cambridge University), which then won the Nobel Prize in

field of Medicine in 1984. Initially, mAbs were murine proteins and were thus immunogenic in humans and not appropriate for chronic therapeutic purpose. In order to produce more human-like mAbs with little immunogenicity, this limitation was overcome using molecular biology and protein engineering. In clinical use and studies, there are many variants of mAbs and traps (Figure 6). Initial attempts substituted most murine Fc sequences with human Fc, a method known as chimerisation. This includes grafting onto a human immunoglobulin (IgG) backbone of the murine antigen binding Fab regions. The mouse hyper-variable peptide binding loops are grafted onto the human IgG backbone in 'humanisation'. More latest technologies allow full human antibodies to be produced.

Figure 6: Monoclonal antibodies. Reducing immunogenicity by chimerisation.

Anti TNF Therapy



Targeting a single pro-inflammatory cytokine, TNF, for the treatment of a complex rheumatoid arthritis (RA) disorder where multiple pro-inflammatory cytokines were upregulated was focused on work by Feldmann and Maini using human disease tissue. They studied the production of cytokines from joints and the control of cytokines in cultures of rheumatoid synovium in which most of the cells survived, creating in vivo mediators. In these cultures, the development of several other inflammatory cytokines (IL-1, IL-6, GM-CSF, IL-8 etc) was reduced by blocking TNF-alpha, thus establishing a 'TNF-dependent cytokine cascade.' The clinical achievement of TNF block, verified first in late-stage rheumatoid arthritis then in earlier stage. In RA, Crohn's disease, ulcerative colitis, psoriasis, psoriatic arthritis, ankylosing spondylitis and juvenile RA, anti-TNF is commonly used; its use is now being investigated in other indications (Shepard et al., 2017). The most effective and commonly used antibody-based therapeutics are anti-TNF antibodies. It is noteworthy that if

used early in the course of RA along with methotrexate, More than 50% of patients can take off infliximab and stay virtually disease-free, even with a decreased dose of methotrexate, and all medication can be withheld from certain patients.

Monoclonal Antibodies in Cancer Therapy

The humanized human epidermal growth factor receptor 2 (HER2) mAb trastuzumab (Herceptin®) has initiated modern mAb therapy of solid tumours. Personalised/biomarker-driven drug development and treatment in oncology was also initiated by the rigorous research that laid the foundation for this breakthrough mAb. Patients are well tolerated with trastuzumab, the first effective monoclonal anti-cancer antibody to be successful against solid tumors. Collaboration between the Shepard (Genentech) and Schreiber laboratories (Chicago) unravelled the pathway leading to TNF resistance of most tumor cell lines, which revealed that macrophages destroy tumor cells primarily by secreting TNF. They speculated that the tumor would become susceptible to killing by host defense if tumor resistance to macrophages could be reversed. Aggressive tumors were developed by macrophage (or TNF)-resistant tumor cells injected into syngenic mice, while their TNF-sensitive parental cells regressed (Shepard et al., 2017).

It has been reported that alemtuzumab (Campath®) – an anti-CD52 and rituximab (Rituxan®) – an anti-CD20) are the first accepted mAbs targeting membrane proteins between haematologic malignancies and their precursor immune cells. Both regular and cancer cells are ablated with these mAbs. Useful in diseases such as chronic lymphocytic leukemia, alemtuzumab was the first humanized mAb therapeutic, depleting lymphocytes, monocytes and dendritic cells. Rituximab similarly depletes CD20-expressing B-cells. As neither CD52 nor CD20 are necessary for malignant development, resistance to these mAb therapeutics occurs with the loss of the target. These mAbs have toxicity problems due to significant immune cell depletion, possibly causing 'cytokine release syndrome' or 'tumor lysis syndrome'-a' cytokine storm' resulting from aberrant immune activation attributable to the activation of 'threat receptors' by cellular debris. Despite these challenges, both rituximab and alemtuzumab are effective medications, frequently leading to disease regression (Shepard et al., 2017).

CONCLUSION:

In conclusion, Ig is the blood substance that is most widely used in clinical practice. Major progress has been made in its development from plasma processing over the years, which guarantees enhanced product protection, in particular with regard to therapeutic uses. Given the different Ig formulations available on the market, they all have to meet the quality criteria that are intended to ensure the quality of this blood product. Using logical design based on experimentally derived or modelled structural details, numerous examples of engineering antibody variable domains have been discussed. To optimize effector function, clustering, and Fc receptor interaction, the Fc region has been engineered. In general, antibody engineering is an indispensable part of the drug development process for both the Fab and Fc regions, and will continue to improve as more and more antibodies are developed for therapeutic use. Despite great success over the last 20 years in antibody engineering techniques, new approaches are in high demand. One of the remaining priorities is to improve the precision of computational methods, which will make it possible to predict point mutations that enhance

affinity and other interesting properties. In order to create antibody-based molecules that are superior in their strength, specificity, localization, and protection, new approaches are continually being created. To engage the appropriate epitopes, the option of the binding domain can be customized. More specific and potent molecules can also result in engineering to modify the architecture of the binding arms, Fc regions, modulatory bispecific, or multi-specific domains to achieve monovalent-or avidity-driven engagement. Therefore, to facilitate comprehensive lead selection, continuous process improvements to generate adequate quantity and purity of hits will be needed. There is still a lot of space for engineering fit-for-purpose "magic bullets" with optimized PK profiles to satisfy various therapeutic hypotheses in the great diversity of antibody structure-function studies.

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