Review

Can cells and biomaterials in therapeutic medicine be shielded from innate immune recognition?

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Biomaterials (e.g. polymers, metals, or ceramics), cell and cell cluster (e.g. pancreatic islets) transplantation are beginning to offer novel treatment modalities for some otherwise intractable diseases. The innate immune system is involved in incompatibility reactions that occur when biomaterials or cells are introduced into the blood circulation. In particular, the complement, coagulation and contact systems are involved in the recognition of biomaterials and cells, eliciting activation of platelets and leukocytes. Such treatments are associated with anaphylactoid and thrombotic reactions, inflammation, and rejection of biomaterials and cells, leading to treatment failures and adverse reactions. We discuss here the new technologies that are being developed to shield the biomaterial and cell surfaces from recognition by the innate immune system.

Innate immune incompatibilities in medical treatments

Innate immunity is fundamental for the defence against microorganisms and foreign substances, and is part of the basis for discriminating between self- and non-self. A number of different systems exist that contribute to this function of which the complement (Box 1), contact and coagulation systems, NK cells, polymorphonuclear leukocytes (PMNs), and monocytes/macrophages represent important components. Cascade systems such as those of complement and coagulation, are in the front line in this defence and are characterized by fast recognition and a powerful amplification. It is therefore natural to find these systems involved in the incompatibility reactions that often occur when alien substances and materials, cells and cell clusters expressing non-self and altered-self structures are introduced into the body using new biotechnological treatment modalities. Procedures involving biomaterial devices, drug delivery systems, viral vectors or cell therapies and cell transplantations, often lead to exposure of biomaterials and cells to whole blood or other body fluids. Treatments with biomaterials have been associated with anaphylactoid and thrombotic reactions, inflammation and, in some cases, infection, leading to treatment failures, adverse reactions and prolonged treatment times [1–3]. New procedures using therapeutic cells have encountered similar problems causing adverse reactions and vast losses of transplanted tissue. The intial phase (recognition) in cell-mediated incompatibility reactions is different but the amplification step is often similar (Figure 1). Therefore, the experience obtained with biomaterials can now be passed on to the cell therapy field.

Recognition and amplification of innate immune reactons on biomaterials

Most biomaterials used in therapeutic procedures are exposed at some time to blood. This applies to biomaterials in extracorporeal circuits (e.g. hemodialysis), biomaterials implanted into the circulation and those implanted into hard and soft tissue (Box 2; Figure 2). Non-biological substances and materials are, in general, recognized by a mechnism that involves adsorption of plasma proteins to the surface exposed to body fluids or blood. Plasma proteins adsorbed to a biomaterial surface, e.g. a polymer, metal, or ceramic, are conformationally changed and thereby 'contact-activated'. Examples of such molecules are IgG (which activates the classical pathway of complement [4]), C3 (which activates the alternative pathway of complement [5]), fibrinogen (which binds to GPIIb-IIIa on platelets) and factor XII (FXII) (which triggers contact activation; Figure 1). The effect of this initial protein adsorption is activation of the coagulation, contact, and complement systems. The subsequent complement activation deposits C3 fragments on top of the plasma protein layer [6]. C3 fragment deposition adds a substantial amount of protein to the initially adsorbed protein mass and can, under favorable conditions, totally cover the original protein layer [5]. After activation by thrombin and the anaphylatoxins C3a and C5a, cells such as platelets, PMNs, and monocytes bind to the protein film on the biomaterial surface.

Adsorbed IgG has been shown to initiate the classical complement pathway on biomaterial surfaces [4] and, even though this activation mechanism deposits only minute amounts of C3b on the surface, the deposition is rapid and sufficient to instantaneously trigger the alternative pathway amplification loop. The relative importance of the different activation pathways is illustrated by in vivo studies, which have shown that in patients with C4 deficiency (who lack the classical pathway) undergoing hemodialysis, complement activation is much delayed, but eventually reaches the same amplitude as patients

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Box 1. Complement activation and regulation

Activation

The complement system has a primary function in innate immunity and host defence by clearing the body of foreign materials and organisms, either by direct lysis or by recruitment of leukocytes that promote phagocytosis. The activation of complement, which consists of approximately 30 plasma and cellular proteins (receptors and regulators), takes place in three steps. (i) Recognition of altered-self or non-self compounds by three different pathways. The classical pathway (CP) is triggered by antigen–antibody complexes, which bind C1q; the lectin pathway (LP) by binding of mannan-binding lectin (MBL) or ficolins to carbohydrates. The alternative pathway (AP) may be initiated by properdin binding to foreign substances, such as microorganisms, or by biomaterials or other surfaces, which do not provide adequate down-regulation of the AP convertase. (ii) Initiation of proteolytic activation of C3 into fluid phase C3a and bound C3b by two multi-molecular enzyme complexes called C3 convertases. (iii) Amplification loop by the AP, which leads to the vast majority of proteolytic C3 activation. The anaphylatoxins (C3a and C5a) activate and recruit polymorphonuclear leukocytes (PMNs) and monocytes to the target, whereas target-bound C3 fragments (C3b and iC3b) facilitate binding to and activation of the recruited cells. The three activation pathways converge into a common pathway forming the membrane attack complex (MAC; termed sC5b-9 in its soluble form) that elicits cell lysis by insertion into the lipid bilayer of cell membranes.

Regulation

The complement system is regulated by multiple soluble and membrane-bound regulatory molecules. Most of these are members of the regulators of complement activation (RCA) superfamily that mainly control the two types of convertases. The plasma proteins factor H (a regulator of the AP) and C4b-binding protein (C4BP; a regulator of the CP), and numerous membrane-bound proteins (MCP [CD46], DAF [CD55], CR1 [CD35]) all belong to this family. All members of this protein family consist of various numbers of homologous short consensus repeat (SCR) domains. In addition, CD59 is a regulator of the sC5b-9 complex and C1 inhibitor (C1INH) regulates the C1 complex.

with normal levels of C4. This reflects the fact that regardless of the recognition mechanism, the alternative pathway amplification loop generates the majority of the C3b molecules that bind to the material surface. In vitro studies have confirmed this mechanism and shown that the majority of surface-bound C3 fragments are iC3b covalently bound to the surface-adsorbed plasma proteins [2,6].

Destructive reactions triggered by innate immunity in cell therapies and transplantation

Clinical cell therapies are emerging treatments for a number of conditions, which include islets of Langerhans (for type 1 diabetes) and hepatocyte transplantation (for hepatic insufficiency), treatment with mesenchymal stem cells (to counter graft versus host disease), and various other stem cell therapies (Figure 2). At present, therapeutic cells are mainly of allogeneic origin (i.e. from genetically disparate human donors), but potential future therapies include xenografts and embryonic stem cells. In many cases, therapeutic cells are infused directly into the circulation, thus exposing the cells to whole blood and all the recognition and immune effector mechanisms this contains. Because most of these cells are foreign and often of non-hematological origin, they are not protected against the cascade systems in the blood.

Clinical islet transplantation is close to becoming an established procedure for treatment of patients with severe type 1 diabetes but the procedure requires that islets from more than one donor are transplanted to achieve insulin independence [7]. In a study reported by Shapiro and colleagues, it was shown that the functional capacity of the transplanted islets from up to four donors to

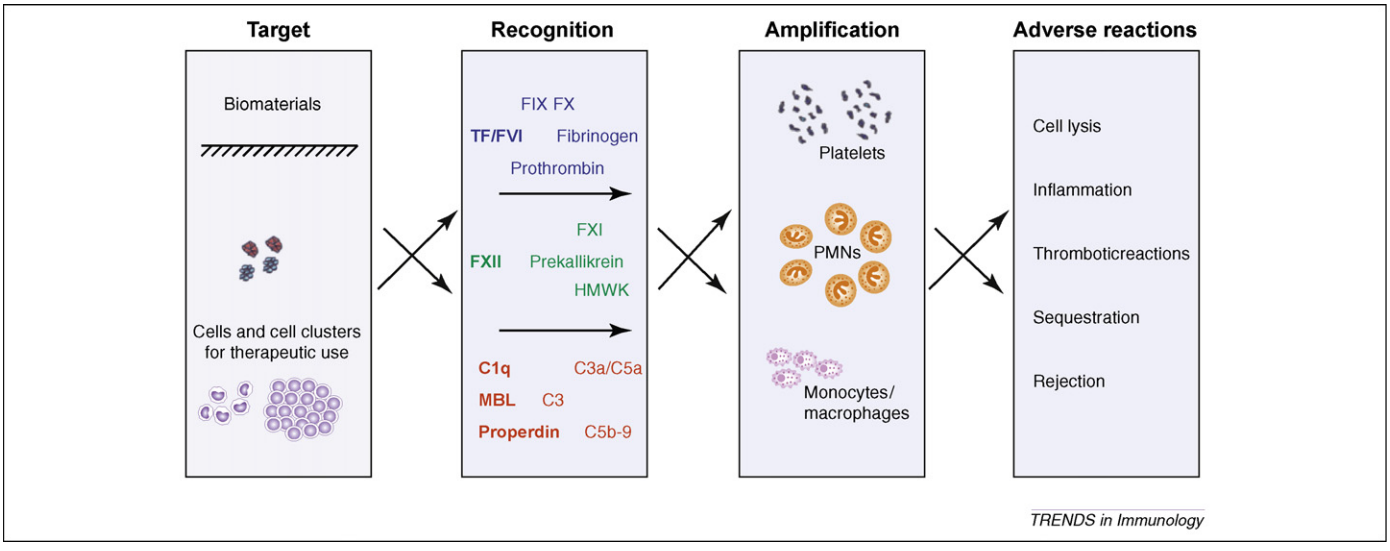

Figure 1. Incompatibility reactions triggered by innate immune responses to altered-self and non-self structures on biomaterials, cells, and cell clusters for therapeutic use. Upon exposure to blood, recognition molecules belonging to different cascade systems target altered-self and non-self structures on biomaterials and cells. Factor (F) VII, fibrinogen, and tissue factor (TF) are 'recognition/trigger' molecules in the coagulation system; FXII and high molecular weight kininogen (HMWK) in the contact system; and C1q, mannose-binding lectin (MBL), and properdin in the complement system. The activation of each cascade system triggers amplification reactions. Activation of the coagulation cascade leads to the generation of thrombin from prothrombin. Further activation of the contact system elicits generation of the potent vasoactive peptide bradykinin from HMWK. In the complement cascade, there is a powerful amplification of C3 that initiates generation of the anaphylatoxins C3a and C5a, as well as the lytic C5b-9 complex. The generated activation products in turn trigger activation of platelets, polymorphonuclear leukocytes (PMNs), and moncytes/macrophages, which result in thrombotic and inflammatory reactions. These adverse events, together with complement-mediated cell lysis and coagulation-mediated sequestration may lead to rejection or serious damage to the biomaterial or transplanted cells.

Box 2. Biomaterials

Biomaterials are materials that come into contact with tissues that are used in therapeutic medicine. There are several types of devices that are used: (1) implants in soft and hard tissue in procedures that include hip replacements, tooth implants, fixation of artificial outer ears etc. (2) Implants in the blood circulation such as heart aids, heart valves, blood vessel prostheses etc. (3) Extracorporeal devices that are used for cardiopulmonary bypass, hemodialysis and plasmapheresis. Typically, the substances used for construction of biomaterials consist of metals, ceramics, and polymers. Metals are often used in joint and tooth replacements and in other types of bone and cartilage devices and steel or titanium alloys are commonly used. Ceramics are used as scaffolds for bone generation. Polymers are the most widely used materials and consist of a variety of different substances, such as hydrogels, plastics, polysaccharides etc. that can be used as scaffolds for vascularization, blood vessel prostheses, drug delivery systems, tubing, artificial hearts, extracorporeal circuits etc.

one recipient corresponded to only 20~30% of that in a non-diabetic individual [8]. Together with the immediate destruction seen during transplantation by positron emission tomography, this indicates that only a small fraction of the transplanted islets engraft successfully [9].

We and others have demonstrated that innate immune reactions may be one of the key factors in destroying the islet graft immediately after transplantation [10–15]. The innate immune response elicits a thrombotic and/or inflammatory reaction, which is triggered when islets come into direct contact with blood in the portal vein. This is known as an instant blood-mediated inflammatory reaction (IBMIR; Box 3) [16]. The IBMIR is a multi-component reaction that is triggered when non-blood cells come into contact with whole blood. In the case of pancreatic islets, complement is triggered by natural antibodies that are preformed antibodies with specificity to a variety of self- and non-self antigens. This is followed by activation of the coagulation cascade elicited by the protein tissue factor (TF) expressed on the transplanted islet cells. Additional complement activation is triggered by binding of C1q to chondroitin sulfate that is released by activated platelets. The islets are finally infiltrated by PMNs mainly due to MCP-1 expressed by the islet cells. Similar reactions occur when hepatocytes and mesenchymal stem cells are exposed to whole blood [17].

IBMIR has been shown to occur in xenogeneic settings when porcine islets are exposed to non-human primates in vivo and to human blood in vitro [18,19]. This type of reaction is, however, more dependent on specific preformed xenogeneic antibodies and complement attack. Future treatment employing human embryonic stem cells (hESC) is likely to mediate similar innate immune reactions, because clinical stem-cell therapy is to be performed with immature progenitor cells that, for example, express the ABO antigens [20].

Shielding against innate immune reactions

Principles for protection against innate immune reactions

From the studies mentioned above, it is evident that inhibition of the contact and complement systems and of clotting (activation of the coagulation system and platelets) is of central importance to improving therapeutic procedures using biomaterials and cells. Heparin is widely used in extracorporeal treatments with biomaterial devices, but we have previously shown that soluble heparin, which is used systemically in clinical cell transplantation, is not sufficient to fully inhibit the IBMIR [16]. A number of drugs

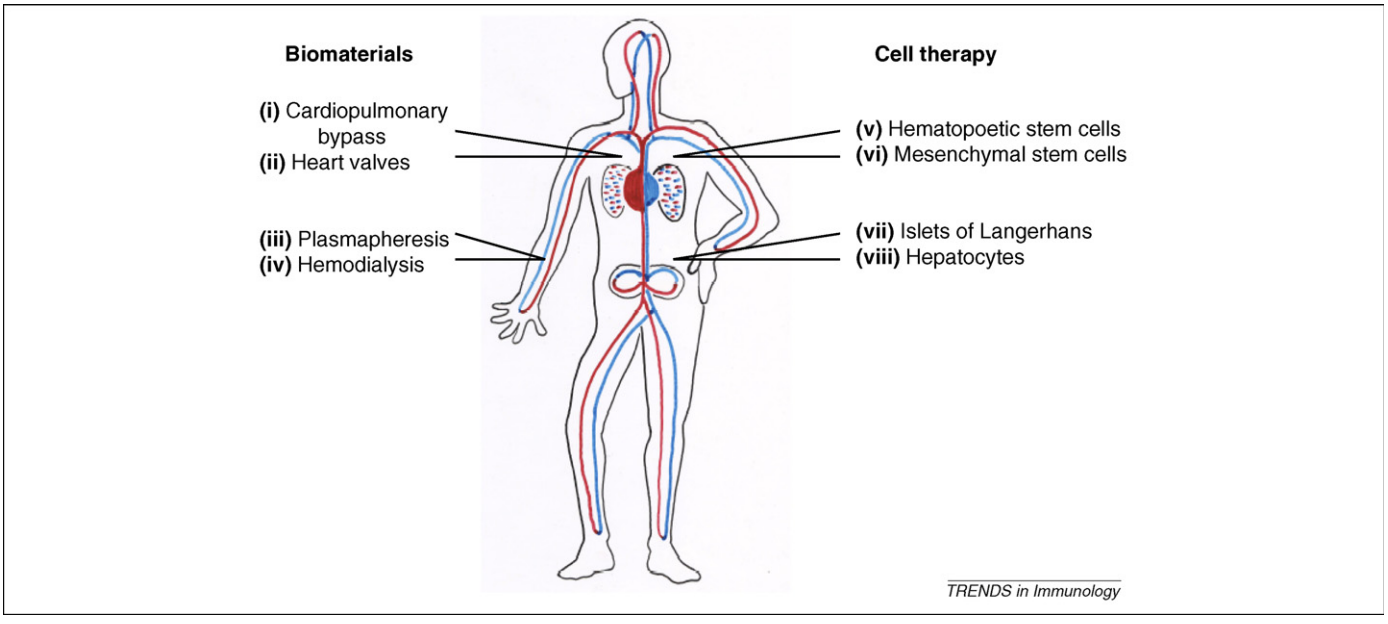

Figure 2. Examples of biomaterials and administered therapeutic cells and cell clusters that come into direct contact with patient blood. (i) During cardiopulmonary bypass, blood is transferred from the patient to an oxygenator where blood gases are exchanged and then returned to the patient. Thrombotic reactions can potentially occur while blood is passing through the extrcorporeal oxygenator and return clots to the patient bloodstream (ii) Heart valves composed of various non-self materials can be in contact with blood for years. (iii) Plasmapheresis is a procedure whereby plasma is prepared from the patient's blood by centrifugation and/or filtration. The plasma may be discarded or processed in an affinity column before it is returned to the patient. Plasma can be drawn from blood donors for therapeutic use. (iv) During hemodialysis, the patient's blood is exposed to a dialysis membrane for many hours several times a week. (v) Transplantation of hematopoietic stem cells and (vi) mesenchymal stem cells is performed by infusing the cells into the blood using a central venous catheter. (vii) Islets of Langerhans and (viii) hepatocytes are prepared from pancreas and liver, respectively, using proteolytic enzymes and are then infused into the portal vein of the recipient.

Box 3. The instant blood-mediated inflammatory reaction (IBMIR)

The IBMIR has similarities with hyperacute rejection (HAR) and ischemia reperfusion in organ transplantation. The IBMIR is a multistep reaction initially characterized in clinical islet transplantation [10–14,16]. Naturally occurring antibodies (IgG, IgM) specific for the transplanted islets trigger complement activation resulting in deposition of components C1q, C4b, C3b and C9 on the islet surface and generation of the fluid-phase activation products C3a and sC5b-9. Leakage of C-peptide (a cleaved product derived from proinsulin) from the islets was reduced by the C3 inhibitor compstatin [44], demonstrating that the islets are damaged by complement attack [45].

Induction of tissue factor (TF) and other inflammatory genes triggers many of the destructive thrombotic and inflammatory reactions that are associated with the IBMIR. In addition to ischemia after procurement of the organ used for cell preparations, the transition to brain death of the organ donor is known to trigger oxidative stress and upregulation of proinflammatory genes [46,47]; e.g. expression of TF [11], and the chemokines MCP-1 [48], IL-8, and MIF [10,49]. TF expression on the transplanted islets triggers activation of the coagulation cascade and the thrombin that is generated elicits platelet activation and fibrin formation, which traps the infused cells in the blood. Due to platelet activation, additional complement activation occurs secondary to the thrombotic reaction. Melagatran, a thrombin inhibitor, attenuates the coagulation activation complements activation despite the fact that this inhibitor has no direct effect on complement components [14]. A mechanism by which activated platelets trigger complement activation is via release of chondroitin sulfate [50]. This glucosaminoglycan binds C1q efficiently and activates the classical pathway of complement.

Other factors contributing to the damaging effects of the IBMIR are that islets have low expression of complement regulators such as DAF (CD55), MCP (CD46) and, to some extent, CD59 [45,51]. This absence of regulators explains the pronounced sensitivity of these cells to complement attack. Also, after preparation and isolation of cells for therapeutic use, which often involves digestion of tissue with enzymes such as collagenase, the cells expose extracellular matrix proteins. Many of these, e.g. collagen, are themselves prothrombotic and act as ligands for binding of platelets to the cell or cell cluster surfaces.

have been identified as potential inhibitors of innate immunity in connection with cell therapies. These include the thrombin inhibitor melagatran [14], different TF inhibitors [11,21], activated protein C [22], and low molecular weight dextran sulfate [23,24]. Systemic administration of inhibitors of the cascade systems of the blood may be associated with increased risks such as bleeding, infections etc. This is a major drawback, particularly in islet and hepatocyte transplantation because these procedures involve transhepatic puncture of a large branch of the portal vein.

Treatment of biomaterials and cells that gives passive protection against attack by the innate immune system

In order to circumvent the deleterious side-effects of systemically administered inhibitors, the biomaterials and cells may instead be pretreated in order to avoid direct contact with selected blood components. The most widely used method is so-called PEGylation (polyethylene glycol coating) of the material or cell surface (Figure 3a). PEGylation increases the hydrophilicity of the biomaterial or cell surface and prevents direct adsorption of proteins, thereby preventing activation of the cascade systems [25]. When applied to cells, PEGylation has the additional benefit of concealing surface structures that would otherwise trigger the activation of cascade systems.

True encapsulation can be achieved by using various kinds of alginate or polysulphone coatings (Figure 3a) [26]. Cells coated in this manner are essentially cut off from the surrounding environment and protected from both humoral and cellular immunity. Polymerization of PEG creates a thinner capsule around the cells and, in addition to the cascade system inhibition, acts as a filter preventing cell contact and the passage of molecules [27,28]. A layered membrane can be formed by PEG-lipid layers through polyion complex formation between amino groups at the end of PEG chains, sodium alginate and poly(L-lysine) [29] (Figure 3a). Such ultrathin membranes embedded with the fibrinolytic enzyme urokinase or the anticoagulant heparin were recently described and should result in greater protection of the cellular graft [30].

The obvious drawback with true encapsulation is deteriorated diffusion of nutrients and oxygen to and from the cells. This is a particular problem with cells in the centre of the larger pancreatic islets, which depend on efficient diffusion in order to survive. Despite this, many groups have reported that both allogeneic and xenogeneic encapsulated islets can survive for long periods (months) following implantation into the peritoneal cavity in both small and large animal models, including in non-human primates [31].

Pretreatment of biomaterials and cells that actively prevents cascade system activation

Heparin coatings have been used extensively as a technique to render biomaterials compatible with blood and its complement, contact, and coagulation systems (Figure 3b). Heparin coatings have been successfully applied to biomaterials such as extracorporeal membrane oxygenators and stents [32]. The accepted hypothesis is that this down-regulation of the innate response is achieved solely by binding of regulators of cascade systems, e.g. antithrombin (AT), C1INH, and factor H, because heparin-bound FXII, and C1, and components of the alternative pathway are reported to be regulated by these inhibitors [32,33]. However, we have found that a striking effect of heparin coatings is that they turn potentially cell-binding biomaterial surfaces into surfaces totally devoid of adsorbed host cells and platelets after contact with blood [34] and we therefore partly challenge the established view of the mechanism by which heparin coatings are 'inert'. We propose that plasma proteins bind to the highly negatively charged, flexible and freely movable heparin-coated surface through both non-specific and specific charge interactions. This binding does not significantly change the conformation of the adsorbed proteins and therefore they fail to be activated. This is supported by the fact that heparin in most cases does not change the function of plasma proteins when they are removed from protein–heparin complexes. For instance, proteins adsorbed to a non-coated surface, IgG bound by heparin will not interact with Fc receptors or C1q, and C3 will not trigger the alternative pathway.

A novel application of heparin coatings has been developed in our laboratory. By using a biotin–avidin–heparin technique, it has been possible to protect transplanted cells (pancreatic islets) from the innate immune response of the

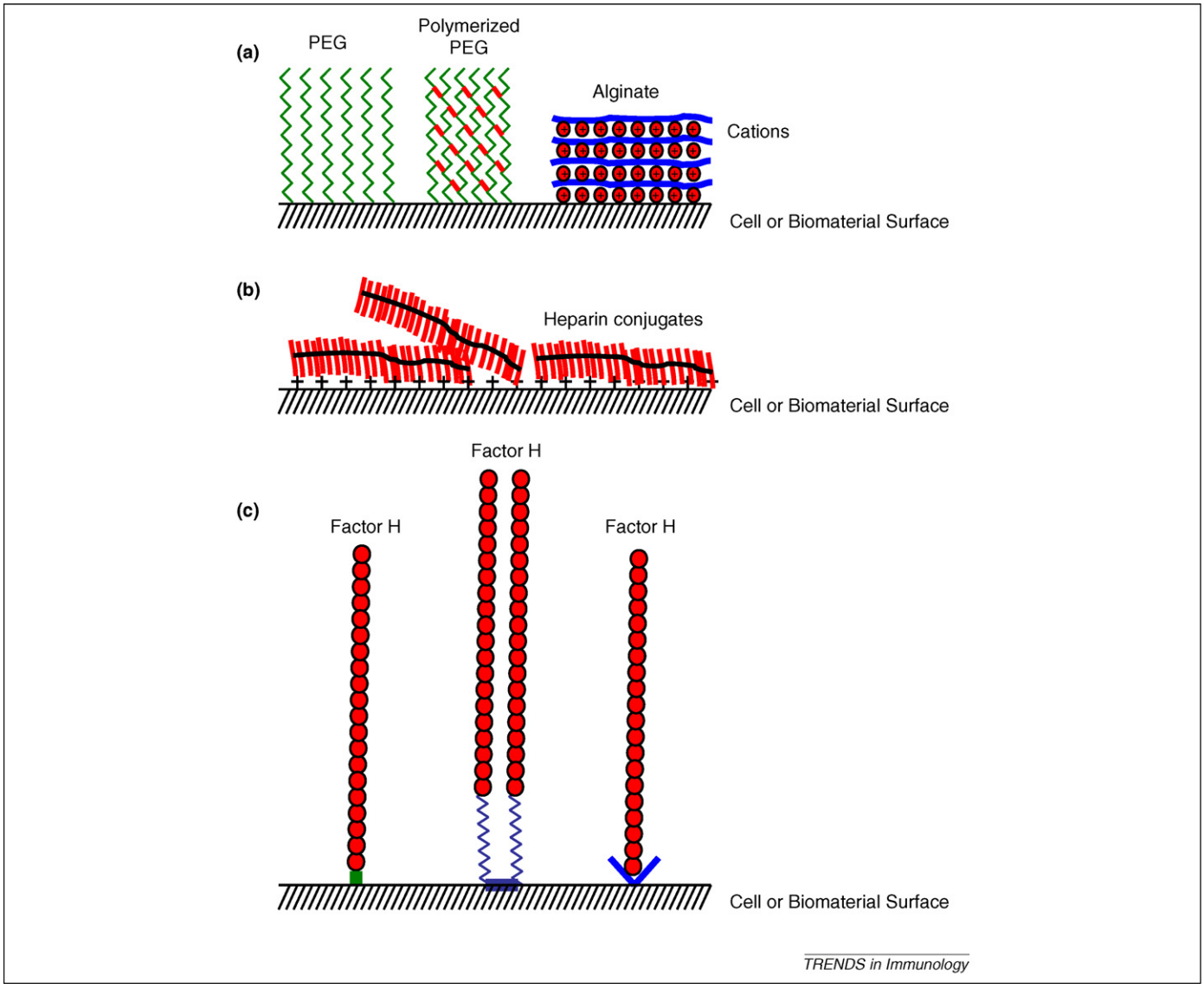

Figure 3. Different strategies to shield the surface of biomaterials and cells against innate immune attack. (**a**) Treatment of biomaterials and cells that gives passive protection against innate immune system attack. Left: polyethylene glycol (PEG) coating (green zigzag); middle: hydrogels consisting of PEG that has been polymerized with a cross-linking agent (red bars); right: a complex between alginate (blue) and cations (red circles). (**b**) Heparin coating consisting of macromolecular complexes of multiple heparin chains (red) covalently bound to a polycarbon backbone (black). (**c**) Different methods for binding a regulatory molecule to a cell or biomaterial surface, exemplified here by the regulator of complement activation (RCA) molecule factor H, consisting of 20 short consensus repeats (SCR). Left: covalent binding using a cross-linking agent (green bar); middle: binding to end group-activated Pluronic®; right: adhesion of endogenous factor H from blood, which binds to a specific peptide, antibody, or antibody fragment (blue V).

blood [35,36]. In vitro, all signs of the IBMIR triggered by the islet cells were abrogated by this heparin coat. In vivo, visible intraluminal macroscopic clotting was found in the portal vein branches of pigs receiving non-heparinized islets, while only scarce thrombi were found in those receiving heparin-coated islets. In the animals receiving heparinized islets, the increase in thrombin–antithrombin complexes and C3a, and insulin dumping due to cell destruction were attenuated, indicating that the IBMIR was reduced. Thus, the heparin coating creates a cell surface that prevents activation of the cascade system without increasing the risk of bleeding and, unlike other pre-treatment procedures, e.g. gene transduction techniques, without inducing acute or chronic toxicity of the islets. In addition, other cell types can be heparin-coated; for instance, hepatocytes, mesenchymal stem cells, and endothelial cells.

Binding of regulators of innate immune reactions to biomaterials and cells

We have assessed the ability of a regulator of complement activation (RCA) i.e. factor H, immobilized on a biomaterial surface, to inhibit complement-mediated inflammatory responses (Figure 3c, left). A cross-linker was used to immobilize factor H on a model biomaterial surface without affecting its biological activity. Immobilized factor H reduced the amount C3b and iC3b fragments deposited on the biomaterial surface after incubation with serum, plasma, or whole blood [37]. In addition, lower levels of the soluble activation markers C3a and sC5b-9 were generated after incubation with whole blood. To explore this effect further, we bound pluronic (a class of triblock copolymers consisting of a block of polypropylene oxide surrounded on each side by polyethylene oxide blocks) to polystyrene surfaces [38]. End-group activated Pluronic® was used

to conjugate factor H to the surface (Figure 3c, middle). Factor H was bound in a physiological conformation and attenuated complement activation on the surface. This created a hybrid surface in which the coagulation-inert properties of the original Pluronic® are supplemented with a specific complement-inhibitory effect.

APT070 (a modified fragment of human complement receptor 1) is an example of an RCA that is bound to cell membranes. This regulator contains the three N-terminal short consensus repeats (SCR) of complement receptor 1 (CR1 also known as CD35) modified with a membrane-targeting amphiphilic peptide based on the naturally occurring membrane-bound myristoyl-electrostatic switch peptide [39]. This regulator is currently being applied in kidney transplantation and could be used in cell therapies [40]. An additional alternative is the newly described fusion proteins consisting of the N-terminal SCRs 1–5 of factor H or DAF (decay accelerating factor), and CR2 (CD21). This creates a molecule that binds specifically to deposited iC3b and C3dg on the material or cell surface and which via the RCA domains inhibits further complement activation [41,42]. Such constructs can be considered for biomaterial and cell surface protection.

Many microorganisms are able to bind the RCA of host complement. For instance, *Streptococcus pyogenes* expresses the M-protein, which binds C4b-binding protein (C4BP), a regulator for the classical and lectin pathways of complement. We have explored a new concept to construct an auto-regulatory surface to inhibit complement activation triggered by biomaterial surfaces in contact with blood (Figure 3c, right) [43]. We coated surfaces with peptides derived from the C4BP-binding region of the streptococcal M-protein. Generation of C3a in the fluid phase and binding of fragments of C3 and C4 to the surface was decreased significantly in serum incubated on surfaces coated with the peptides as a function of their C4BP-binding capacity. Using Streptococcus-derived peptides we could provide proof of principle for the auto-regulatory concept; i.e. that it is feasible to capture complement regulatory molecules from plasma to control autologous complement activation at a model biomaterial surface. This and all of the above-mentioned techniques are applicable on cells.

Concluding remarks

The use of biomaterials and cells, and combinations thereof, in therapeutic medicine is becoming increasingly important. Many of these constructs come into direct contact with whole blood, whereupon an innate immune response causes inflammation, destruction of implanted cells, and impaired biomaterial performance. Therefore, to achieve safer and more efficient therapeutic interventions it will be necessary to engineer both cells and biomaterials that can avoid these adverse reactions. Both passive and active shielding will need to be used in the future to protect bioengineered constructs. Conjugation of biomolecules to biomaterials and cells that can regulate innate immune reactions are promising concepts, which avoids the potential cell-damaging and pro-inflammatory effects of transduction vectors. Medical device technology includes numerous potential applications for cross-linkers and spacer molecules that are capable of specifically binding biomolecules to surfaces while retaining their activity. These biomolecules can be RCAs or other innate immunity regulatory molecules. In the future, auto-regulatory compounds capturing endogenous regulators should be constructed as peptides with low molecular weight and immunogenicity. Progress has been achieved with biomaterials with regards to biocompatibility and innate immunity reactions and much of this knowledge can be transferred to the cell therapy field. However, much progress still remains to be made in order to understand the mechanisms by which different transplanted cells trigger innate immune reactions and how these reactions can be attenuated.

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