Introduction to Stealth Nanoparticles

Module Objective

This module provides information on the importance of avoiding immune recognition for a drug delivery system. At the end of the module, the learner will be able to appreciate the strategies to modify the nanocarrier so as to avoid immune recognition.

Preface

In vivo efficacy of a delivery system depends upon its residence time in the body. However, the various components of the immune system recognize the nanoparticles as foreign bodies and activate pathways to eliminate them from the system. An understanding of the various routes of immune activation can provide a solution to avoid immune recognition. This forms the crux of this module. The various factors that influence the stealth characteristics are also discussed in this module.

This lecture discusses in depth the necessity of escaping immune recognition for drug delivery systems. It also describes the process of opsonization that serves as the first step in immune recognition of nanoparticles.

1 What is Stealth?

One of the major research areas in nanomedicine is in the development of nanocarriers to deliver drugs to specified locations. Though *in vitro* experiments employing the drug-loaded nanocarriers are successful, the efficiency of these drug carriers *in vivo* is not encouraging as they fail to reach their target. What could be wrong? There are numerous factors that can influence the efficiency of a drug carrier*in vivo*. These include the route of administration, preferential uptake by non-target cells (biodistribution) as well as the lifetime of the carrier within the biological system.There are many options for introduction of the nanocarrier into the biological system such as through nasal, transdermal, parenteral, oral, rectal or ocular routes. The efficiency of the system is different in each case. Why? The carrier molecule faces different types of barriers and filters in each case before it can enter the blood. As a result, the amount of drug-loaded carriers that enter into the blood circulation varies with the route of administration. Once it is in the blood, the carrier faces an entirely new challenge! Blood contains an arsenal of components that form part of the body's defense – namely the immune system. The nanocarrier is not native to the biological system and is considered a foreign body by the immune system.Hence, it is either degraded or eliminated from the biological systemas soon as the carrier is recognized by the immune system. As a result, the drug present in

the nanocarrier never gets to reach its target! Therefore the carrier needs to escape recognition by the immune system components! The properties of the nanocarrier have a major influence on the response of the immune system as well as its interaction with other non-specific targets in the biological system. These include the particle size, surface charge, surface topography and hydrophilicity. As a result of such non-specific and undesirable interactions with non-specific targets as well as the immune system, the carrier and the drug loaded in the carrier do not get delivered to the target. Therefore, suitable modifications must be made to the nanocarrier to impart the ability to 'hide' from the immune system. ***The ability of a nanoparticle to evade immune recognition so as to enhance its circulation timein vivo and thereby its chances of reaching the target is known as 'stealth characteristic'.***

One of the earliest attempts employed to evade the immune recognition was to flood the system with a placebo carrier before introducing the drug-loaded carrier. A placebo carrier does not contain any drug. The high concentrations of the placebo carrier will activate the immune components, which will then be engaged in eliminating these carriers. If the drug-loaded carrier is introduced at this time, the chances of it getting recognized and eliminated by the immune system is remote as the immune components are involved in destroying the placebo carrier. This diversionary strategy unfortunately comes with a price! The very high concentrations of the placebo carrier introduced into the system can cause undesirable side effects owing to the disruption of the immune system. Hence this strategy has been discarded.

Introduction of immune suppressive agents is another strategy that tends to disable the abilities of the immune system. Though this strategy is used in extreme cases, it is not desirable as the patient becomes vulnerable to other infections during this period. In recent years, a '*Trojan horse concept*' has been employed to impart stealth characteristics to the nanocarrier to enable it to escape from immune recognition without causing any impairment to the immune system.

The origin of the Trojan horse concept…

In Greek mythology, a long war was fought between the kingdoms of Troy and Greece. The Greeks laid siege to the city of Troy for more than 10 years and still no end to the war was in sight. Hence, they decided to win the war by employing a stealth strategy. They constructed a large wooden horse with a secret compartment in which they hid some of their soldiers. Then they pretended to sail away making the Trojans think that the Greeks had given up. The unsuspecting Trojans took the horse inside their city to mark their victory. In the night, the soldiers inside the horse came out and opened the gates of the city to let in the Greek army, which then plundered the city and won the war! In the case of delivering the drugs to selected locations in the biological system, a similar strategy has to be employed to trick the immune system. The carrier becomes the Trojan horse carrying the 'soldiers', namely the drug molecules. The immune system fails to recognize the danger posed and once the carrier enters the 'city of Troy', which in this case is the target cell, the drug molecules come out of the carrier and destroy the cell. Hence the Trojan horse concept is very relevant in nanomedicine to design stealth carrier systems!

In order to devise strategies to impart stealth characteristics to the carrier, one has to understand the pathways leading to immune recognition of foreign bodies. The response of the blood components to the nanocarrier will be of prime importance in deciding the fate of the nanocarrier in the biological system. Apart from the nature of the nanocarrier, the flow parameters of the blood also influence the interactions between the blood and the carrier. These include the kind of stress experienced by the carrier surface owing to the flow of blood – static, laminar or vortex, and the ratio of the blood volume in contact with the nanocarrier surface. Any carrier that does not elicit much response from the blood components is termed as '**hemocompatible**'. The search for the ideal hemocompatible material is still on. Apart from the natural vascular endothelial cells lining the inner side of the blood vessel, no material can be classified as 100% hemocompatible!

2 Immune recognition pathways

One of the first events that occur when a nanocarrierenters the blood stream is '**opsonization**'. Blood consists of a huge number of proteins and ions. When the nanocarrier comes into contact with these components, adsorption of the blood proteins on the surface of the nanocarrier occurs. This is a rapid process and is **diffusion-controlled** in the initial phases. This means that the first interacting components will be the hydrated ions followed by the more mobile proteins. Among the ions, it has been found that the divalent calcium and magnesium ions are the most active. The later stages of opsonization are **affinity-controlled** as the larger proteins that have greater affinity to

the nanocarrier surface displace the weakly adsorbed species. The protein adsorption process over the nanocarrier is thus a competitive process and the sequence of proteins that adsorb on the surface and then on each other is described by '**Vroman effect'**. The major blood proteins involved in the adsorption process are albumin, globulin, fibrinogen, fibronectin, Factor XII and high molecular weight kininogen. These proteins are referred to as the '**Opsonins**'.

What is the consequence of this protein adsorption? Well, the opsonin proteins can interact with the receptors in the cells of the mononuclear phagocytic system (MPS) that form part of the cell-mediated immune response. This interaction leads to the activation of the monocytes and macrophages,which results in the uptake the nanocarrier by the macrophages or activationof the other components of the immune system. The nanocarrier is taken up by the macrophage by forming a vesicle termed as phagosome. This then fuses with the lysosome containing an array of hydrolytic and degradative enzymes that destroys the nanocarrier. This process of uptake of the carrier by the macrophages or other components of the MPS is termed as '**phagocytosis**'.

Why is this process termed 'opsonization'? The term 'opsonin' is derived from the Greek language and means 'seasoning' or 'sauce'. Similar to the process of seasoning food items with herbs, spices or other ingredients to improve its flavor and edibility, the nanoparticle surface is coated or 'seasoned' with proteins to improve its uptake and digestion by the cells of the MPS! Hence this sequence of events is referred to as opsonization. Figure 1 shows a cartoon depicting the sequence of events occurring during opsonization.

Why should opsonization drive phagocytosis? Can't the macrophage phagocytose a foreign body without opsonization? The immune system has evolved mainly to combat and eliminate pathogens. Most pathogens have an outer membrane that is predominantly negatively charged. Unfortunately, the macrophages and the other cells of the MPS also possess a negatively charged cell membrane. Hence uptake of the pathogen by the macrophages is retarded due to the existence of greater electrostatic repulsive forces. Hence, the immune system has evolved an effective strategy of coating the surface of the pathogen with the opsonin proteins that will mask the surface charges of the pathogen thereby facilitating uptake and subsequent degradation by the macrophages.Though it is not necessary that all nanoparticle surfaces will be negatively charged or even charged at all, the process of opsonization is the first they will encounter when they enter the blood stream.

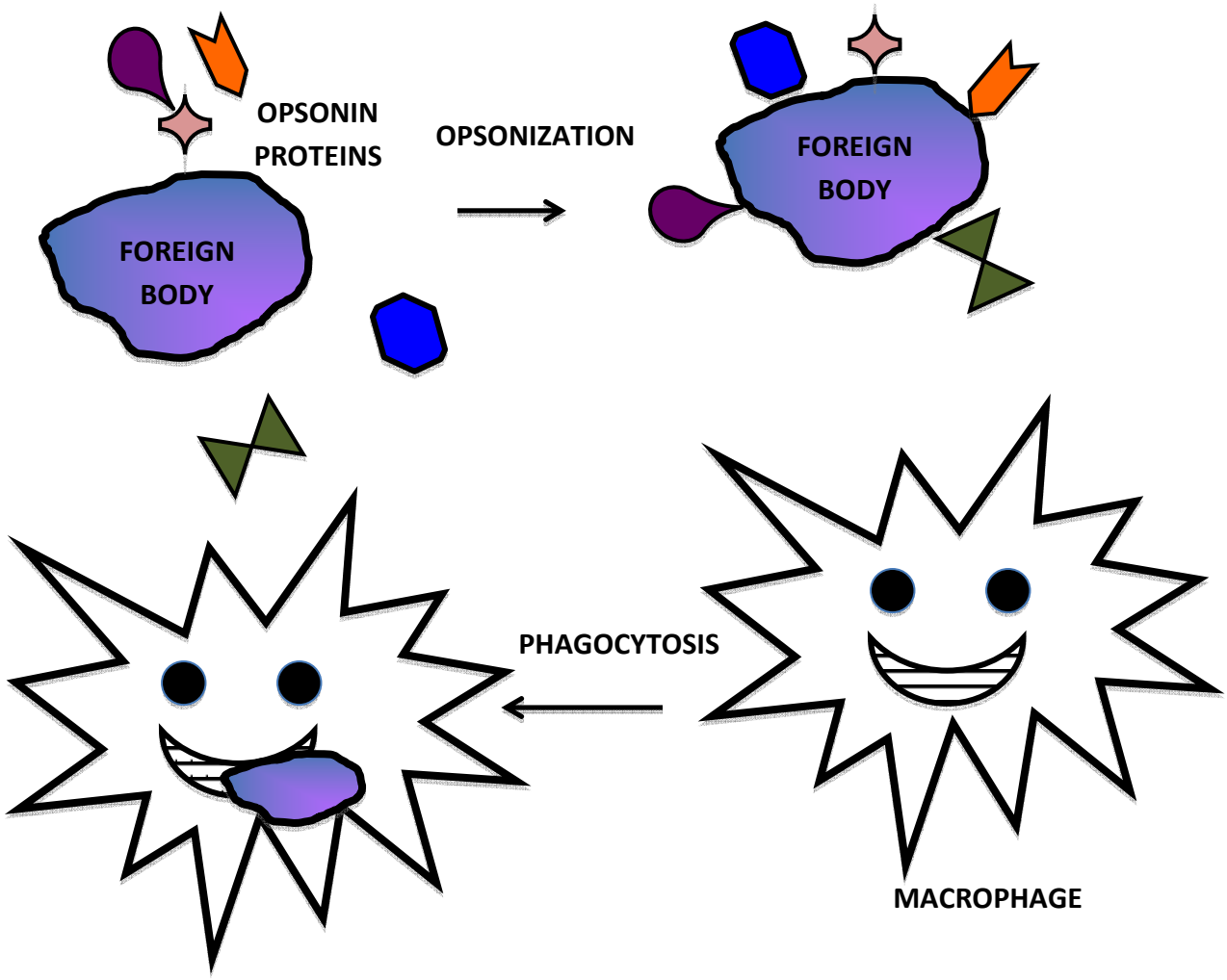

Fig. 1: Cartoon showing the sequence of events during opsonization

What will happen if the nanocarrier could not be degraded by the MPS? In this event,the entire carrier molecule or part of it is anchored on the cell surface and 'presented' or exhibitedto the external environment by the MPS cells using major histocompatibility (MHC) proteins. This results in the activation of another component of the immune system, namely the T-lymphocytes, which in turn activate the B-lymphocytes leading to production of antibodies against the foreign body.

Among the range of opsonins that can adsorb on to a foreign body surface are a group of proteins called as the complement proteins. If the complement proteins adsorb on to the surface of the nanocarrier, then another cascade of events leading to the activation of the

complement system is also triggered. The mode of activation of the complement proteins is elaborated in the following lecture.

3 Reference

Encyclopedia of Nanoscience& Nanotechnology, Volume 9. Edited by: H.S. Nalwa, American Scientific Publishers, 2005

4 Additional Reading

Smart Nanoparticles in Nanomedicine (The MML series, Vol. 8), Editors: Reza Arshady& Kenji Kono, Kentus Books, 2006