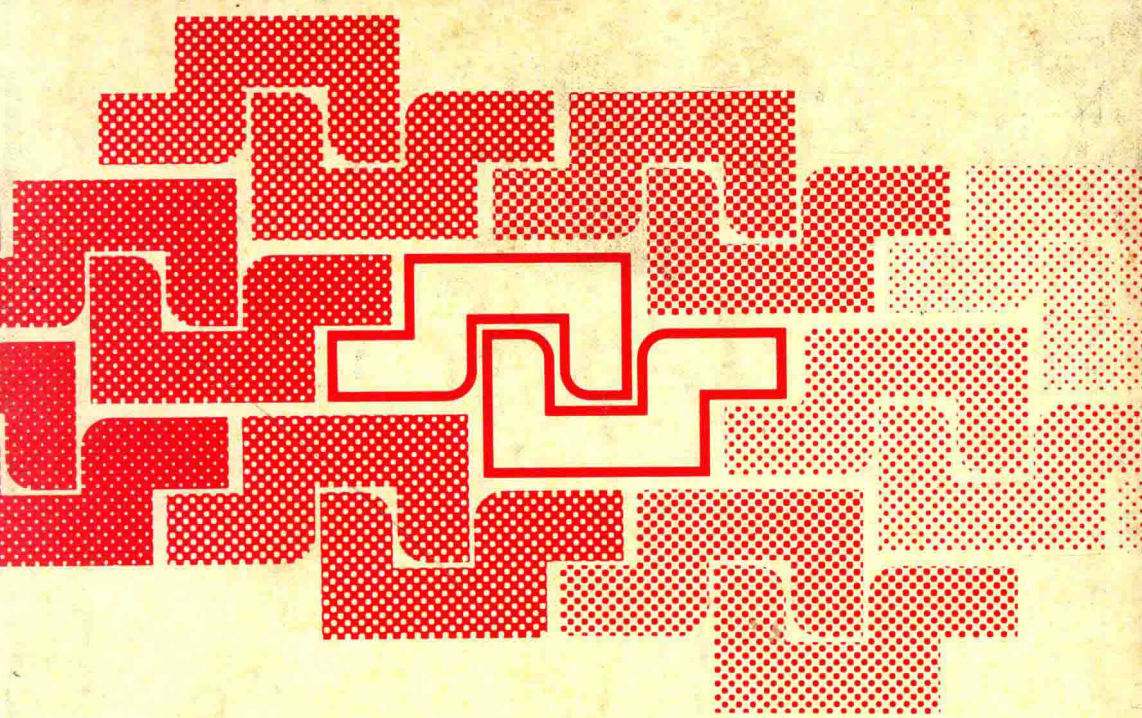

Radiolabeled Cellular Blood Elements

Pathophysiology, Techniques, and Scintigraphic Applications

Edited by

M. L. Thakur

NATO ASI Series

Series A: Life Sciences Vol. 88

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Pathophysiology, Techniques, and Scintigraphic Applications

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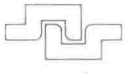

Series A: Life Sciences

PREFACE

This book is based on the contribution made by the guest lecturers and some of the participants gathered at the Advanced Study Institute (ASI) on Radiolabeled Cellular Blood Elements held in Maratea, Southern Italy, August 29, to September 9, 1982, under the auspice of NATO. The first such international symposium on this subject was held in New York in September 1979, the proceedings of which were published by Triverum Publishing Company in New York in 1980. Since that period we have witnessed an ever increasing number of publications and investigations of the subject of radiolabeled cellular blood elements throughout the world. The time was right, therefore for this ASI to bring together experienced investigators, present and potential users, and promising young scientists for exchange of knowledge and experience, informal dialogues, and relaxed and fruitful discussions.

The guest faculty included veterans who placed emphasis on basic cell physiology in health and disease, upon which numerous applications of radiolabeled blood cells are based. Presentations were also made by key investigators who have contributed for many years, outlining problems and potential solutions and giving a critical look at a variety of techniques used and applied in vivo. Unlike the research articles in the proceedings of the first symposium therefore this monograph contains chapters on basic cell physiology and critical reviews of years of data and experience generated in the preparation and use of radiolabeled blood cells. In addition to the main chapters by the guest lecturers, the appendix contains a few short papers on important contributions made by a few of the participants. Unfortunately many could not be included owing to lack of space.

Preparing manuscripts with figures and tables for camera reproduction was a formidable task. Care has been taken for consistency and typographic accuracy. However, I make no claim that no errors exist in this volume. I hope, however, that the reader will understand and ignore any error and find this compendious volume useful for numerous biologic studies, physiologic explorations and clinical applications of radiolabeled cellular blood elements in years to come.

M. L. Thakur
Editor, and the ASI Director

ACKNOWLEDGEMENTS

Dr. Max Hardeman of the University of Amsterdam and Dr. Michael Ezekowitz of Yale University served as the codirectors of the Advanced Study Institute (ASI). Dr. Hardeman spent countless hours and contributed to the scientific program, took care of mailings in Europe, organized transportation in Italy, and communicated with the hotel management on numerous occasions. I cannot thank him enough!

Dr. Ezekowitz collected some manuscripts and corrected a few. I am grateful to him.

I take this opportunity once again to thank all the guest faculty, who, despite their busy schedules and time constraints, accepted my invitation and made valuable contributions to the ASI. I am also grateful to all participants, who were so friendly and were primary resources for many lively discussions. They made the ASI professionally beneficial and socially enjoyable.

Dr. diLullo, Dr. Sinclair, and Mrs. Kester of the NATO Division of Scientific Affairs in Belgium were especially helpful from the beginning of the ASI. Without their advice and understanding, this ASI could not have been so successful.

The hotel manager, Mr. Guzzardi, and his staff treated us well for two weeks. I thank them all on behalf of all of us.

It is with deep appreciation that I acknowledge the supplementary financial assistance we received from Medi-Physics, California; Amersham-International, England; Mallickrodt Inc., St. Louis, Missouri; and Beckman International, Amsterdam.

I also want to express profound gratitude to Mr. John Curry and his staff particularly, Elmina Finck, Ann Marie Webster, and Amy Yee of the American College of Radiology, Philadelphia, for the help I received in administrative and financial aspects of the ASI and in the long dragging task of correcting, typing, and retyping the manuscripts for camera reproduction.

The help I received from Patricia Vann and her editorial staff at Plenum Publishing and from my secretary, Debbie Bronson, is also appreciated.

CONTENTS

PATHOPHYSIOLOGY, BIOCHEMISTRY, AND PHARMACOLOGY OF PLATELETS

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INTRODUCTION

This subject will be presented in relation to the study of platelets and vascular disease. Platelets are involved not only in thrombosis but also in atherosclerosis, which is a key factor in the development of arterial thrombosis. The study of arterial disease and thrombosis in living subjects has been difficult.

Attempts to study vascular injury, vascular disease, and thrombosis in experimental animals and humans are limited because of our inability to monitor these processes in living subjects effectively. The development of methods for imaging internal organs and for detecting the deposition of radioactively labeled materials have made it possible to monitor some of these processes in living subjects.

Detailed reviews of the points presented in this paper can be found in the reviews listed in the References(1-9).

Among the aspects of vascular disease that could be assessed by imaging techniques are the following:

1. atherosclerotic lesions,
2. endothelial injury,
3. spasm,
4. thrombosis,
5. embolism.

ATHEROSCLEROSIS

Monitoring atherosclerotic lesions will probably require techniques such as NMR, doppler ultrasound, and positron emission tomography as well as angiographic techniques to provide effective ways of studying the progression and regression of atherosclerotic lesions. Relatively little can be done to study atherosclerotic lesions using labeled platelets, although deposition of labeled white blood cells, particularly monocytes, might be an indicator of some forms of active atherosclerosis where monocytes are playing a key role in the development of the lesions.

ENDOTHELIAL INJURY

Endothelial injury can be an initiating event in the development of atherosclerosis, in arterial spasm, and in the mechanisms involved in the formation of arterial thrombi. Therefore, a way of studying endothelial injury and endothelial alteration in intact living subjects would be desirable. At present there are no established methods for studying this, although there are some possibilities to which I will refer. Principally, these focus on the detection of products formed and secreted by the endothelial cells which may reflect stimulation or injury, for example, von Willebrand factor and plasminogen activator, and the effects of products from stimulated endothelium on blood constituents such as platelets and possibly white cells, that could influence their turnover in the circulation.

SPASM

The role of spasm in inducing the clinical complications of atherosclerosis is still controversial, but during the past decade an overwhelming body of evidence has developed that indicates that coronary artery spasm does occur, particularly in relation to variant angina, and that this can be a factor causing coronary ischemia. Spasm tends to be associated with advanced atherosclerotic disease and appears to occur most frequently at sites of extensive narrowing of the vessels as a consequence of atherosclerosis. Studying this process is difficult because the only reliable method that can be used at the present time in man is coronary artery angiography. Some of the newer techniques may, of course, make it possible to study spasm without having to inject contrast media.

THROMBOSIS

Thrombosis can be studied by some of the imaging methods and more specifically by studying the accumulation of radioactive constituents of the thrombi. The accumulation of iodine-125-labeled fibrinogen and possibly other proteins such as plasminogen, chromium-51 or indium-111-labeled platelets or chromium-51 or indium-111-labeled leukocytes can be examined. This is obviously a major focus of this meeting.

EMBOLISM

We are less successful in studying the fragments of thrombi that break off and embolize into the distal circulation. However, as I shall try to point out, this is an extremely important event that needs an approach that will allow studies in living subjects.

The next section of this paper outlines the processes that are involved in the initiation and development of vascular disease and its thromboembolic complications with particular emphasis on platelets and the implications for any in vivo monitoring techniques.

ENDOTHELIAL INJURY

Perhaps the most important point to be made in relation to endothelium is that platelets do not interact with normal endothelium; scanning electron microscopy of a normal aorta shows no platelets adherent to the endothelium. In contrast, white cells do interact with both normal and altered endothelium. The nature or importance of this white cell interaction with the endothelium has not been established, but there is some evidence that the white cells, such as polymorphonuclear leukocytes and macrophages, may be able to cause endothelial cell alteration and even loss of the endothelium and that macrophage accumulation on the subendothelium can contribute to the development of atherosclerosis. Recently it has been shown that platelets can adhere to foam cells formed from macrophages in atherosclerotic lesions when the endothelium covering the macrophage is lost.

VESSEL INJURY AND THROMBOSIS

When the endothelium is lost from the surface of the vessel, platelets generally form a thin layer over most of the surface where blood flow is mainly laminar. The adherent platelets appear to be in contact with collagen, basement membrane, and the microfibrils associated with the elastin in the subendothelium. Platelets adherent to collagen discharge their granule contents and the arachidonate pathway is activated leading to TXA_2 formation. If von Willebrand factor is available, platelets adherent to the microfibrils also undergo a release reaction. In areas where blood flow is not disturbed, no thrombi accumulate and only a single platelet layer adheres to the surface of freshly damaged normal arteries. Obviously the accumulation of platelets on a freshly injured surface could be detected using isotopically labeled platelets. However, two factors can influence this: the number of platelets that accumulate and the duration of reactivity of the injury site.

Within 30 minutes of the initial formation of the platelet layer on the subendothelium the platelet-covered surface is largely nonreactive. Platelets are gradually lost from the subendothelium,

during the first few days after loss of the endothelium. The surface that is exposed to the circulating blood is nonreactive to circulating platelets and does not activate the coagulation process. Thus, in studies of injury in arteries, if the radioactive platelets are given before the injury occurs, radioactive platelets will accumulate on the surface and may possibly be detected by gamma scanning. However, if the radioactive platelets are given after the injury has occurred, they will not displace the initial layer of nonradioactive platelets that has formed on the surface and therefore it is very unlikely that one will be able to detect the injury sites by infusing radioactive platelets.

At present, we do not know what makes these vessels nonreactive, but we do know that it is not PGI_2, and it is probably not related to the adsorption of plasma proteins onto the surface of the vessel.

When a normal vessel is injured with a balloon catheter, platelets accumulate on the subendothelium and release their granule contents, including the platelet-derived growth factor (PDGF), which is mitogenic for smooth muscle cells. Smooth muscle cell migration and proliferation leads to a thickening of the vessel wall within seven days following removal of the endothelium. The surface of this smooth muscle cell intimal thickening exposed to blood is nonthrombogenic.

When a diseased vessel or previously damaged vessel is injured, the process that occurs on the surface is different from that which occurs on the freshly exposed subendothelium of a normal blood vessel. When the neointima is damaged, platelet-fibrin thrombi form on much of the surface of the vessel. In some areas there are few thrombi, but these surfaces are covered with a platelet layer such as seen on the subendothelium. Thus, the interaction of the constituents of the blood with a damaged neointima appears to be more complicated than with the subendothelium. Results from a number of experiments indicate that in these circumstances, platelet-fibrin thrombi are probably produced by activation of the coagulation mechanism, with resultant thrombin generation. The thrombin induces both fibrin formation and platelet aggregation, and a complicated thrombus forms in which the platelets are not in contact with connective tissue of the vessel wall but are mainly in contact with fibrin which is adherent to the vessel wall. The formation of these platelet fibrin thrombi can be inhibited by heparin, but, in contrast, the adherence of the platelets to the subendothelium is not influenced by the doses of heparin that block the formation of the platelet fibrin thombi on the injured neointima. The injured neointima behaves in a similar manner to the exposed subendothelium. That is, the platelet-fibrin thrombi are lost from the surface of the vessel over the next 24 to 48 hours and there is little fresh platelet or fibrin accumulation on the surface.

Again, assessment of thrombosis at such injury sites will be dependent upon the radioactive material being given before the injury occurs. If radioactive material is given after the injury, relatively little radioactive material will accumulate at the injury site, making detection of the injury site difficult. Thus, we can conclude from studies in experimental animals that thrombus formation in arteries, regardless of whether the mechanism initiating the accumulation of thrombi is mediated by connective tissue or mediated by thrombin, is a limited process that will be difficult to study using radioactive materials unless the materials are present before the injury occurs or there is repeated or continuous injury to the vessel wall.

In contrast to vessel walls, prosthetic grafts show a different behavior toward platelets. On the basis of the published evidence it appears that many prosthetic surfaces tend to remain reactive to circulating platelets and coagulation for a long period of time after their insertion. Thus the administration of radioactive platelets to subjects or animals with prosthetic grafts will detect platelet accumulation on the surface over a long period of time. On some of these grafts there is considerable fibrin formation as well, and therefore the process can be monitored by examining the accumulation of radioactive platelets or radioactive fibrinogen on the grafts or measuring platelet survival which may be shortened.

We should keep in mind that a nonthrombogenic surface can be an endothelial surface, a smooth muscle cell surface, a subendothelium covered with the initial platelet layer after it has undergone the initial reaction, the subendothelium after the platelets have been lost from the surface, or a damaged smooth muscle cell layer after the initial reaction has occurred. Thus, it is not surprising to find ulcerated atherosclerotic lesions with little evidence of thrombus formation on them.

If one examines the carotid artery bifurcation where atherosclerosis commonly occurs and where thromboembolic events are believed to initiate in many subjects, one can find a range of morphological appearances. There may be advanced atherosclerosis with thrombosis, advanced atherosclerosis without ulceration or thrombosis, or advanced atherosclerosis with ulceration and no thrombosis. At present we do not know what makes a surface of an ulcerated lesion nonthrombogenic. The study of arterial thrombosis is enormously difficult and since at present we have no simple way of predicting when an injury will occur that will initiate thrombosis, it is difficult to monitor thrombotic processes by means of circulating radioactive materials. It is important to keep in mind that this differs to some extent from venous thrombosis. When a thrombus occurs in a vein, the thrombus tends to persist for a considerable period of time and the fibrinogen in the thrombus turns over, making it possible to give radioactive fibrinogen and demonstrate its accumulation in the thrombus.

Occlusive thrombi in arteries tend to occur in areas where there is marked narrowing of the blood vessels in association with disturbances in blood flow. As such sites, the shear stresses may not only injure the wall but obviously augment platelet interaction with the wall and with each other. If marked narrowing is produced in a normal coronary artery, platelet aggregates will form spontaneously and may occlude the lumen for short intervals at such sites. Under these circumstances, it is possible to observe repeated formation and dissolution of platelet thrombi. Obviously the formation and dissolution of these thrombi could be monitored by the use of radioactive platelets. It is not entirely clear what occurs at such sites, but two points are important. First, when the rate of blood flow is greatly increased, it creates high shear rates that can disrupt the endothelium. This tends to occur in areas where the lumen is very narrow and induces strong movement of platelets to the vessel wall, leading to the rapid formation of platelet aggregates. This is probably one of the main mechanisms that is involved in hemostasis in small blood vessels. Thus, at stenotic lesions, it is possible that the effects of flow could induce endothelial injury and the formation of platelet thrombi and platelet-fibrin thrombi. Presumably these sites will remain at risk for thrombosis as long as the flow effects cause platelet accumulation and/or endothelial injury caused by the flowing blood occurs repeatedly. Thus one could have situations in which there is frequent and repeated induction of the thrombotic process as a result of hemodynamic forces. It should be possible to detect such thrombi with isotopic techniques. Areas in the human arterial tree that are exposed to conditions that could allow for the study of recurrent thrombotic episodes are a stenotic carotid artery and areas of stenosis in the peripheral arteries of the lower limb particularly at bifurcations and branches involving the femoral and popliteal arteries where marked atherosclerotic narrowing occurs.

SPASM AND THROMBOSIS

When a vessel is injured, it goes into spasm. This is well documented in studies of hemostasis. Thus, it is not surprising that arteries with advanced atherosclerosis can go into spasm. It has been demonstrated that the smooth muscle cells that proliferate in the intima following endothelial injury appear to be more sensitive to agents such as thromboxane A_2 and serotonin that induce spasm than the smooth muscle cells in the media. The problems here are how to study spasm in relation to ischemic events, and how to assess its contribution to thrombosis.

When spasm occurs it can alter blood flow and can facilitate thrombus formation. Spasm may cause sufficient narrowing to produce some ischemia and then the vessel may relax, allowing normal blood flow to resume. However, if a thrombus is formed, it may continue to block the lumen of the blood vessel after the vessel relaxes. Alternatively, the thrombus may be dislodged when blood flow resumes