Significance of Immunological Studies in Peripheral Obliterating Vascular Diseases

By Josef Pokorny, M.D., C.Sc., and Zdenka Ježkova, Ph.D., C.Sc.

In angiology, many efforts have been made to find methods which might be helpful in the differential diagnosis of vascular diseases. These include clinical, biochemical, histological, immunological, and many other methods. In searching for a method to give us at least partial confirmation of a differential diagnosis, we turned to antivascular antibodies. The purpose of our investigation was to facilitate the diagnosis of the nature of vascular lesions by means of observations of immunological processes which may reflect changes in the blood vessel wall.

Two basic assumptions were made: (1) antivessel antibodies do not occur in normal human beings, and (2) protein or lipoprotein carriers of immunological material resulting from pathological processes can act as heterogeneous substances and lead to the formation of corresponding antibodies. These problems have only recently begun to attract attention.1-5 The aim of our work was to find out (1) if antibodies against pathologically changed blood vessels actually exist, (2) if they exist as a unit or are formed against individual blood vessel layers, and (3) if there are quantitative differences in the antibodies against individual layers of the blood vessel coat.

Methods

The method was a complement test and has already been described.6 As an antigen, we used normal as well as atherosclerotic vessels obtained freshly at operations and after injuries. The vessels were either frozen at -20 C. until enough tissue was obtained for the preparation of antigen or were treated immediately in the fresh condition, as described below. Individual layers were separated by dissection. The whole blood vessel or its individual coats was homogenized in a blender and suspended in 30 parts of saline solution. We used 0.1 ml. of this suspension in each reaction. To examine one patient, 1 ml. of active patient’s serum is required. Serum is divided into two parts, 0.5 ml. each. Then, 0.1 ml. of vessel antigen (already diluted 1:30) is added to the first tube, and 0.1 ml. of saline solution is added to the second, which is the control. Both tubes are placed in an incubator for one hour at 37 C.

Meanwhile, two series of 12 test tubes are prepared, containing decreasing volumes of saline solution, as outlined in table 1. After one hour of incubation, 9.5 ml. of saline solution are added to both tubes containing 0.5 ml. of patient’s serum. After thorough shaking of both tubes, gradually increasing volumes of the diluted examined serum are pipetted into the 12 saline tubes, so that each tube contains 1 ml. The diluted serum without antigen is similarly pipetted into a second series of 12 tubes. Finally, 0.25 ml. of hemolytic system, containing four units of amboceptor, is added to each tube, and after thorough shaking the entire lot is placed in a water bath at 37 C. for 15 minutes.

Reaction evaluation: The first test tube with complete hemolysis serves as a basis for evaluation of results. If the results of this first test tube with complete hemolysis and those of the row containing antigen differ by more than 2 test tubes, the result is rated +, if by 3 test tubes ++, if by 4 test tubes ++++, and if by 5 or more test tubes ++++.

Results

The first phase of our study dealt with the presence of antivascular antibodies in healthy blood donors. In 200 healthy subjects, using this complement consumption test (CCT), we found only exceptionally a slightly increased titer of antibodies (+), the maximum being 1 per cent.

A high titer of antibodies against antigen from normal blood vessels or from vessels changed by atherosclerotic processes was found in patients with acute phlebitis migrans (table 2). A significantly increased titer of antibodies against normal as well as pathologically changed blood vessels was present.
TABLE 1

<table>
<thead>
<tr>
<th>Scheme for the Titration of Complement*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
</tr>
<tr>
<td>Serum examined 1/20</td>
</tr>
<tr>
<td>Hemolytic system</td>
</tr>
<tr>
<td>Test tube number</td>
</tr>
</tbody>
</table>

*The serum is diluted 1/20, and 0.15 to 0.80 ml. is pipetted into the test tubes.

in 31 patients with phlebitis migrans, while in 145 young patients with thromboangiitis obliterans without phlebitic signs, it was present only in one case.

Antibodies in patients with phlebitis migrans disappeared gradually as the inflammation subsided, and after treatment with hydrocortisone, prednisone, or pyrabutol (Irgapyrine), they disappeared in a few days. They were again found during recurrence of acute phlebitis (fig. 1).

Our results (table 3) show that the occurrence of antibodies against antigen from the blood vessel wall is statistically significant in phlebitis migrans as compared both with the obliterative phase of thromboangiitis and with the group of healthy donors, of whom only 0.1 per cent gave positive reactions. It is noteworthy that antibodies against antigen from both normal and atherosclerotic arteries are not demonstrable in superficial venous inflammations other than migratory phlebitis. In only a single case of deep ileofemoral phlebitis were the results positive.

In 145 cases of atherosclerosis obliterans, we found 51 per cent positive for antibodies against the whole vessel wall as well as of antibodies against its individual coats. The increased titer of antibodies existed either against all three coats of the vessel wall or at least against two of them. These three layers are never affected to the same extent (table 4). Thus, in acute phlebitis migrans, we observed a very high titer of antibodies in atherosclerosis obliterans might be diagnosed as progressive development or activation of an atherosclerotic process. Such conclusions, however, will remain tentative until they are validated by more extensive data, including complete histological and histochemical examination of biochemical findings and possible changes of the blood vessel wall.

As a further check on the method, antibody neutralization tests were also carried out with serum saturated with an excessive amount of antigen from normal arteries and then centrifuged; part of the serum was also examined against sclerotic arteries. We also tested the inverse order of antigens. In both series of tests, sera gave positive reactions even after saturation with the second antigen.

To determine whether antivascular antibodies occur against all vascular coats, we examined adventitia, muscularis, and intima from some patients who showed increased titer of antibodies against the whole vascular wall as well as of antibodies against its individual coats. The increased titer of antibodies existed either against all three coats of the vessel wall or at least against two of them. These three layers are never affected to the same extent (table 4). Thus, in acute phlebitis migrans, we observed a very high titer of antibodies against antigen from adventitia and muscularis. In the chronic form of the same disease, anti-adventitia antibodies were absent, anti-muscularis antibodies were positive (++), and anti-intima antibodies were also positive (+++). In atherosclerosis, antibodies were demonstrated against the whole blood vessel wall; a more detailed analysis, however, showed a higher occurrence of anti-intima and anti-media antibodies in comparison with anti-adventitia antibodies.
In those cases in which no antibodies against the blood vessel as a whole were present, we were unable to demonstrate antibodies against any of its coats.

**Discussion**

The high incidence of antibodies against blood vessel wall in patients with thromboangiitis obliterans is interesting and may be important, but in the present state of immunology, it is not possible to determine from these findings whether the increased titer of antibodies against the arterial wall is solely the result of a process caused by a metabolic disorder of the blood vessel wall, or whether it is partially influenced by tissue ischemia, gangrenous process, or even an associated lymphangitis. Our finding antibodies against the individual layers of the blood vessel wall indicates that the increased titer of antibodies most probably arose from pathological processes in the blood vessel wall. The occurrence of such antibodies might serve as a criterion of the extent of pathological changes of a certain constituent of the blood vessel wall; the result of these changes is the occurrence of antigens resulting in antibody response.

However, negative results against some constituents of the blood vessel wall do not exclude its antigen properties, since it is difficult to determine whether the profound antigen change took place in some of the immunologically examined coats of the blood vessel wall or whether the titer of antibodies against it was not so high as the titer of antibodies against the other coats, so that these antibodies were not detected by the method employed.

On the basis of our examinations of antibodies against the arterial wall, we demonstrated a statistically significant difference between the sera of patients with phlebitis migrans in the course of thromboangiitis and those with the obliterative phase of this disease. A different immunological picture was obtained in patients with atherosclerosis obliterans, where the increased titer of antibodies was found in a considerable percentage of cases. It was true, especially in cases in which progressive tissue ischemia was accompanied by trophic disorders, gangrene, or lymphangitis. So far, very few reports dealing with immunological studies of atherosclerosis have been published, though the follow-up of antibodies in these cases could help in the detection of various reactions of individual subjects to both internal and external stress factors of the blood vessel wall.

In patients with atherosclerosis—in contrast to thromboangiitis—the question must be considered whether the findings of high titer of antibodies against the arterial wall do not

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**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Positivity</th>
<th>Negativity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromboangiitis</td>
<td>4</td>
<td>144</td>
</tr>
<tr>
<td>Migr. phleb.</td>
<td>31</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>177</td>
</tr>
</tbody>
</table>

$\chi^2 = 159.2 > 10.8 \ldots \ldots .01$ per cent.

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Positivity</th>
<th>Negativity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migr. phleb.</td>
<td>31</td>
<td>199</td>
</tr>
<tr>
<td>Blood donors</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>232</td>
</tr>
</tbody>
</table>

$\chi^2 = 204.3 > 10.8 \ldots \ldots .01$ per cent.
mean the reactivation of the atherosclerotic process. On the contrary, negative findings in atherosclerotic processes can also indicate a deficiency of reactivity resulting from insufficient formation of antibodies. The aim of our report is to call attention to the wide scope of these problems.

Attention should also be drawn to the fact that an elevated titer of anti-arterial antibodies was found in patients with coronary sclerosis in whom myocardial infarction developed. The antibodies were demonstrated about the tenth day of acute myocardial infarction.

On the basis of our results, we conclude that, from the clinical point of view, it is useful to study anti-arterial antibodies in the acute phase of thromboangiitis obliterans, where they are important for differential diagnosis. They indicate disorders of the blood vessel wall, perhaps the existence of other processes as well.

In atherosclerosis, before definitive conclusions can be drawn, further study and control examination of enzymatic, biochemical, and histological processes will be necessary to provide a satisfactory explanation of our findings. We conclude, however, that the finding of a higher titer of antibodies can indicate the activation of the atherosclerotic process and therefore has prognostic value.

Immunological studies of vascular diseases, combined with biochemical findings, might help to disclose many processes affecting the blood vessel wall or other tissue of mesenchymal origin, even of organs abundantly supplied by the blood vessels, and perhaps may warn of a future breakdown of body defenses in the course of organic blood vessel diseases such as atherosclerosis.

TABLE 4
Antibodies Against Arterial Wall*

<table>
<thead>
<tr>
<th>Possibilities</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Adventitia</td>
<td>++++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Media</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Intima</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

*Various possibilities of positivity or negativity of antibodies to the layers of the blood vessel wall are shown. If antibodies to the blood vessel as a whole are found, the following possibilities can occur:

1. Strongly positive findings in acutely affected arteries (usually of inflammatory origin).
2. The most frequent findings in acute phlebitis migrans (i.e., in the acute phase of thromboangiitis obliterans).
3. This column shows the most frequent findings in the chronic form of thromboangiitis obliterans (i.e., without any findings of acute phlebitis). In this, antibodies to adventitia of arteries can be negative, while anti-muscularis and anti-intima antibodies can be positive. Similarly, in atherosclerosis obliterans (where positive antibodies to the whole blood vessel were found), the detailed analysis resulted most frequently in positive findings of antibodies to intima and media as compared with antibodies to adventitia.
4. It is shown that in cases where no antibodies to the whole blood vessel were found, antibodies to individual layers were not found either.

Summary
A statistically significant incidence of antibodies against antigen from normal arteries and blood vessels changed by an atherosclerotic process was found in patients in the acute phase of thromboangiitis obliterans. No such incidence was found in a control group of healthy blood donors and in patients with other diseases. These findings of antibodies can significantly help in the differential diagnosis of the acute phase of thromboangiitis obliterans. It is possible that estimations of antibodies may also be of some value in patients with atherosclerosis obliterans where especially the neutralization test could be important for differential diagnosis of atherosclerosis.
PERIPHERAL VASCULAR DISEASES

Acknowledgment
We are indebted to Mrs. Alena Majdá for her valuable technical assistance.

References

Book Reviews

This is the English translation of the monograph which appeared in Hungarian seven years ago. All aspects of the lymphatics are covered: history of the discovery, phylogenesis, ontogenesis, and anatomy. The formation of lymph and its absorption are adequately discussed. The special physiology and pathology of the lymphatic system in the heart, lungs, gastrointestinal tract, and kidneys are excellently reviewed.

This atlas consists of 42 sample tracings of intracardine and intrapulmonic pressures derived from a variety of patients. The legends for each figure were originally written in German and translated in English and Spanish. The opening introduction of A. Command is excellent.

This monograph is intended to discuss all important aspects of the syndrome: anatomy of the veins, pathology of thrombosis, pathogenesis, diagnosis, symptomatology, treatment, prevention, and rehabilitation. The book has been written for the physician who desires a summary of new concepts and improvements in therapy. The illustrations are limited in number, but the excellent text material overcomes the necessity for any more.

This volume marks the first of a comprehensive review of the physiology of the cardiovascular system. Each of the 22 chapters in this book was written to fit the needs of three groups of readers: (1) the graduate student who wants to go more deeply and broadly into the meanings of current physiological concepts and their background than he can in standard text books; (2) the teacher who is dissatisfied with the comprehensiveness of his understanding outside his own specialty; and (3) the investigator who will use it as a springboard for references and current concepts in a field which he is beginning to explore.
Like other similar ventures, the quality of the chapters is not uniform. In this first volume, the opening chapter by C. J. Wiggers is highly recommended, not only to the groups specified above, but to anyone interested in circulation research.

The 38 papers presented at the recent International Symposium organized by the British Occupational Hygiene Society are included in this book. The toxicology of the lungs has been systematically covered: anatomy and physiology, physical and chemical aspects of particle retention, radioactive aerosol, particle-vapor interaction, pulmonary elimination and storage of dust, asbestosis, and pneumoconiosis. Most of the articles serve as a review of the present literature of the particular topic.
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