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Immunofluorescence studies in recent myocardial infarction

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Authors' synopsis *Immunofluorescence studies (IF) were carried out in 60 patients with a recent myocardial infarction and with three control groups, comprising respectively 30 patients with acute or chronic coronary insufficiency, 16 with various heart diseases, and 60 healthy subjects. Circulating anti-heart antibodies were detected in 19 of the 60 patients with a recent myocardial infarction; in two cases with Dressler's post-infarction syndrome their titre was as high as 1:160. In one case of myocardial infarction with a high antibody titre there were no signs of the post-infarction syndrome. This patient was on corticosteroid treatment for other reasons. In the control groups immunofluorescence was positive at the dilution of 1:10 in only one of the 106 subjects.*

Autoimmune processes in heart diseases, especially in those involving myocardial necrosis, are the subject of numerous studies (Kaplan, 1958; Dressler, 1959; Van der Geld, 1964; Faivre, Gulgen-krantz, Duheille, and Petitier, 1967).

The results obtained with various methods differ very widely. The use of the immunofluorescence method for the detection of circulating antibodies directed against heart antigens has yielded results which appear to be reproducible in different laboratories. With the aid of this method, Van de Geld (1964) demonstrated anti-heart antibodies in the serum of eight out of 13 patients with Dressler's post-infarction syndrome. It is important to note that he also observed positive IF staining in cases of recent infarction without accompanying symptoms of the post-infarction syndrome.

Faivre *et al.* (1967) used the indirect immunofluorescence method to demonstrate antiheart antibodies in 17 of their 34 cases of recent myocardial infarction. It should be noted that they detected them in all five cases with Dressler's post-infarction syndrome.

The purpose of the present studies was to investigate the frequency with which anti-heart antibodies occur in patients with recent myocardial infarction and to compare the dynamics of the immunological phenomena with the clinical course.

Subjects and Methods

Our subjects included 60 patients with recent myocardial infarction; their ages ranged from 33 to 73 years (average 54 years) and they were treated in the department for internal diseases between 10 January 1967 and 6 June 1968. In each case, the diagnosis was based on unambiguous clinical and electrocardiographic patterns, enzyme studies, and other basic laboratory investigations.

There were 106 control subjects: (1) 30 cases of coronary insufficiency, including 11 acute cases; (2) 16 cases with other heart diseases (pericarditis with effusion – three, rheumatic carditis – four, valvular disease – five, so-called thyrotoxic cardiopathy – three, and idiopathic pericarditis – one; and (3) 60 healthy subjects aged 20–61 years (average 41 years).

The immunofluorescence methods (IF) used were the indirect method of Weller and Coons (1954) and the mixed labelled-antigen method of Beutner, Holborow, and Johnson (1965). The sera were taken within 24 hr after the infarction and then weekly throughout the period of hospitalization (about six to eight weeks). In patients with positive results, the IF tests were repeated monthly, in some of them until the results became negative.

The antigen was rabbit heart tissue frozen in liquid nitrogen and kept at a temperature of −20°C for not more than two weeks. Sections, 4 μ thick, were prepared in a cryostat (−20°C); they were dried for 20 min at room temperature before use.

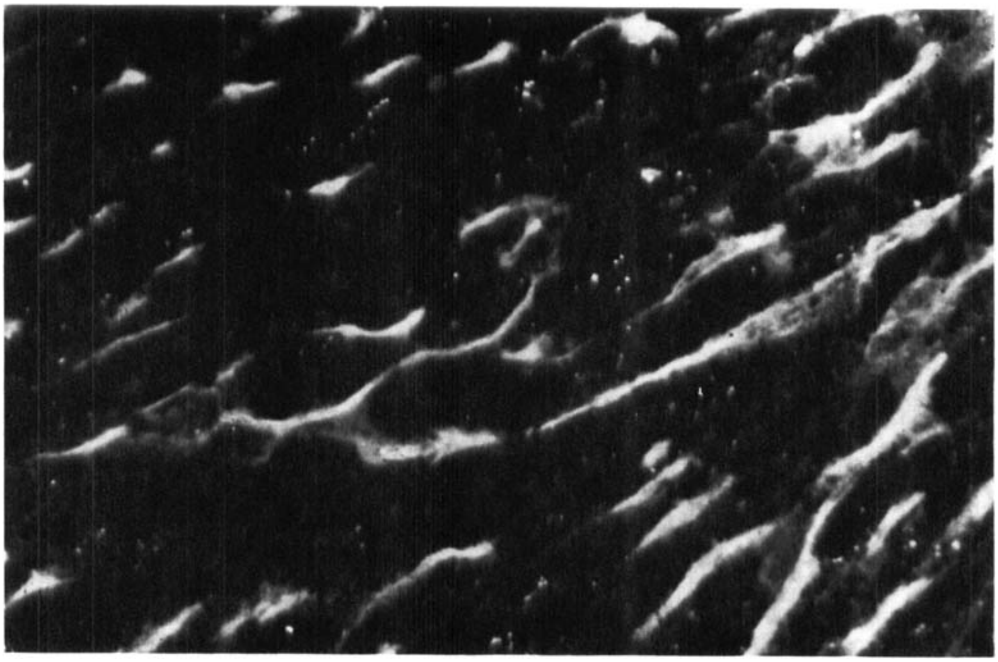


FIG. 1 *Heart infarct. Labelled antigen – IF staining. Rabbit's heart tissue as a substrate. Note specific fluorescence of the muscular structures on the cell periphery (subsarcolemma).*

Indirect IF method

(Weller and Coons, 1954; Beutner and Jordon, 1964; Chorzelski, Weiss, and Lever, 1966; Chorzelski, Jabłońska, Błaszczyk, and Jarząbek, 1967). The tissue sections were placed on a slide and incubated for 30 min at room temperature in a wet chamber with the investigated serum diluted 10-fold with phosphate buffered saline at pH 7·2. In order to remove the uncombined immunoglobulins, the sections were rinsed twice for 15 min in buffered saline. They were then dried and covered with rabbit immune serum directed against human IgG labelled with fluorescein isothiocyanate. The resulting conjugate has the following characteristics after the method of Marshall, Eveland, and Smith (1959), and Beutner *et al.* (1965): 16 u./ml. F/P 4·83, dilution 1:120–⅛ u./ml. (Strauss, Kemp, Vannier, and Goodman, 1964). The immune serum was prepared by immunizing rabbits with human IgG isolated on a DEAE cellulose column. In immunoelectrophoresis the immune serum gave with normal human serum a single arc in the IgG zone.

The preparations were incubated 30 min, and then rinsed in phosphate buffer under the same conditions as in the first stage of the reaction.

Labelled antigen mixed IF method

(Beutner *et al.*, 1965). Sections were incubated with the serum under investigation and rinsed in phosphate buffer and covered with unlabelled rabbit immune serum against Cohn Fraction II of human serum (16 u./ml. diluted 1:4 to 4 u./ml.). Preparations were then incubated for 30 min again and washed twice for 15 min. Lastly, the preparations were incubated with Cohn fraction II of human serum (1 mg/ml.) labelled with fluorescein isothiocyanate (F/P not determined) and then washed to remove the uncombined part of the fraction.

The preparations were sealed in buffered glycerol under a cover slip and examined in a fluorescence microscope (Fluorolume American Optical Company) with a dark field condenser and UG 5 plus BG 12 primary or exciter filters.

In order to judge the specificity and reproducibility of the results, a positive control was used each time – that is, the serum of a case of myocardial infarction with a known anti-heart antibody titre was used in parallel tests. For negative controls the sera of healthy subjects and isotonic saline were used.

For checking the immunological specificity of the results we used the so-called blocking method – that is, negativization of the immunofluorescence reaction. In the indirect method this was achieved by substituting unlabelled immune serum directed against human IgG for the labelled one. In the labelled-antigen indirect method the labelled antigen (fraction II) was replaced by an unlabelled one of the same kind.

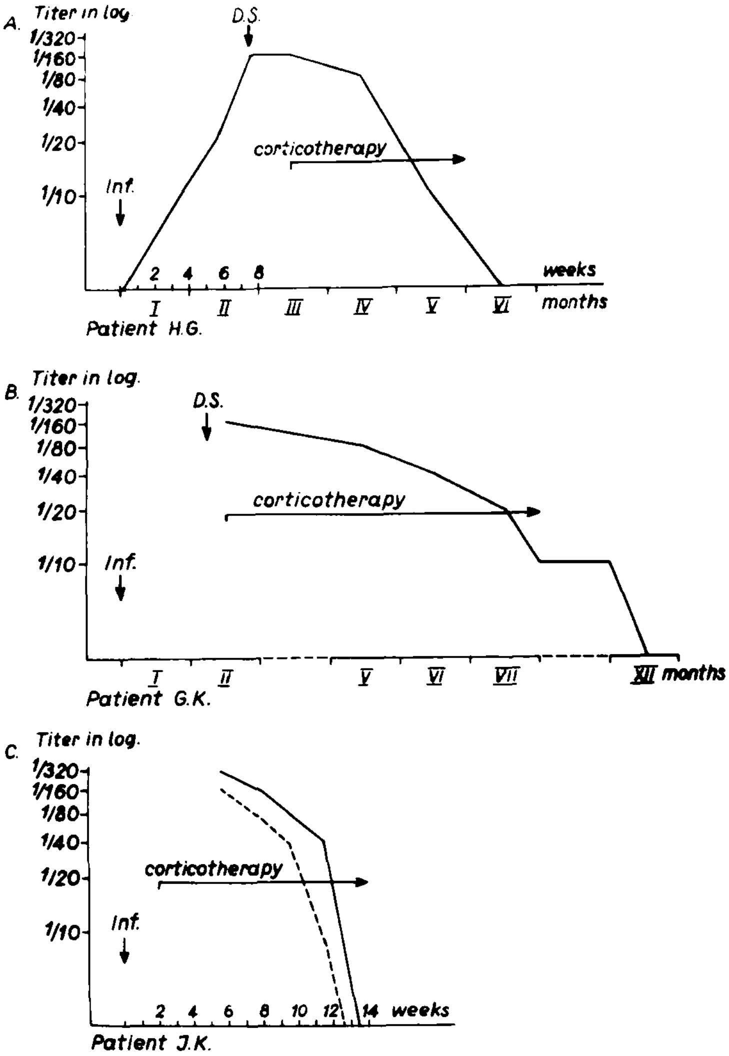
Results

In the first 10 cases both indirect and mixed IF methods were used. They gave comparable

TABLE I *Immunofluorescence investigations in ischaemic heart disease*

Diagnosis	*Number of cases*	*Positive fluorescence*
Recent myocardial infarction	60	19*
Chronic and acute coronary insufficiency	30	1
Other heart diseases	16	0
Healthy subjects	60	0

* One patient had a titre of 320 and two 160 respectively. In 16 patients the titre ranged from 10 to 20.



results, but the microscopic picture was clearer with the labelled antigen mixed IF method. Thus the latter method was used throughout the rest of this study.

The labelled-antigen, mixed IF method gave positive results in 19 of the 60 cases of recent myocardial infarction (Fig. 1) – that is, in 31·7%.

Among the 106 controls, one gave a weakly positive result; it was from a patient with chronic coronary insufficiency. In all the other cases, the results with control sera were negative. The results recorded in particular groups are shown in Table 1.

Dressler's post-infarction syndrome developed in two of the 60 cases of recent myocardial infarction (patients H.G. and G.K., Fig. 2A and B). The IF results were positive in both, with the sera diluted 1:160. In 16 of the 17 remaining positive sera, all had lower titres (see below). In one of the strongly positive cases (patient H.G.) the results called attention to the character of the post-infarction complication and the case deserves to be reported in greater detail.

H.G., a man aged 61 years, was admitted to the clinic for recent infarction, a history was given of a myocardial infarction four years previously. During the fifth week in the clinic there was a new infarction, and a week later there appeared in the left lung symptoms which could correspond to inflammatory changes, with an effusion reaction in the pleura. The changes resisted sustained comprehensive anti-inflammatory treatment, showed some radiographic variability, and eventually involved also the right lung and pleura. Old tuberculous changes in the upper lobes as well as adhesions and bronchiectases at the base of both lungs made radiological analysis initially difficult. IF tests made within the first six weeks at the clinic were negative. During stormy clinical symptoms, the titre of antibodies was found to be as high as 1:160; together with the clinical symptoms, this finding warranted the diagnosis of the post-infarction syndrome (see Fig. 2). Cortisone treatment was fairly rapidly effective in liquidating the pleuro-pulmonary complications.

FIG. 2 *Titres of anti-heart antibodies as revealed by indirect labelled antigen – immunofluorescent staining. A = patient H.G. with Dressler's syndrome; B = patient G.K. with Dressler's syndrome; C = patient J.K. with pemphigus and atypical myocardial infarction. DS = Dressler's post-infarction syndrome. Inf. = myocardial infarction.* ——— *anti-heart antibodies.* – – – *pemphigus antibodies.*

TABLE 2 *Immunofluorescence investigations in recent myocardial infarction*

Diagnosis		*Number of cases*	*Positive fluorescence*	%
Recent myocardial infarction	first	38	9*	23·7
	second and next	22	10†	45·5

* The titre ranged from 10 to 20.
† In three patients the titre ranged from 160 to 320 (see Fig. 2) and in seven patients the titre ranged from 10 to 20.

In one patient (J.K.) who did not suffer from the post-infarction syndrome, the titre of anti-heart antibodies was very high – 1:320 (Fig. 2C). This patient was treated two weeks after the infarction with large doses of prednisone (100 mg daily) for pemphigus (Chorzelski and Kuch, 1969) (Fig. 2).

In the remaining 16 cases, the titre of antibodies was low (1:10 to 1:20). The clinical course in these patients varied from mild to very severe and was not significantly different from that in cases with negative IF tests. However, none of these cases suffered from Dressler's syndrome. Anti-heart antibodies appeared in the blood between two weeks and six weeks after the infarction and persisted for several weeks. Antibodies persisted longest in cases with Dressler's syndrome – namely, nine months in one, and about one year in another.

Tabulation of positive IF tests (Table 2) shows clearly that antibodies directed against the heart are found more commonly in patients who have already had an infarction before the most recent one.

Discussion

Immunofluorescence tests for antibodies directed against the heart yield reproducible results when suitably standardized reagents are used. In our studies we used two methods: Weller and Coon's indirect IF method and the labelled-antigen mixed IF method introduced by Beutner *et al.* (1965). We found that results with the latter method were particularly clear and completely free of non-specific effects.

The proportion of positive results – 31·7% in 60 patients with recent myocardial infarction – approaches the somewhat higher figure recorded by Faivre *et al.* (1967) – 50% in 34 patients – who used the indirect method.

In two of the 19 cases (patients H.G. and G.K.) with positive IF results, we were able to relate the clinical picture with immunological processes. These were Dressler's post-infarction syndromes with the titre of antibodies as high as 1:160 during the climax of clinical symptoms and then persisting for a long time (about one year) at a lower level.

It seems that establishment of the presence of anti-heart antibodies does not by itself prove their pathogenetic role. It is possible that their high level and persistence is connected with the complication (Chorzelski and Kuch, 1969) described by Dressler (1956, 1959) as the post-infarction syndrome.

It is conceivable that what is involved in the pathogenesis of the post-infarction syndrome is the antigenic necrotic protein referred to as alloprotein (Pór, Milar, Madar, Sokol, Koppel, and Koziak, 1967) whereas the antibodies merely testify to the fact of an infarction.

The higher incidence of positive immunofluorescence tests in cases in which the recent infarction is not the first one may be explained by the anemnestic response – that is, immunological 'recall' phenomenon or secondary response seen in animals which are being immunized.

Our attention was attracted by the fact that some of the patients with positive IF tests develop various allergic conditions, such as bronchial asthma, drug allergy, and eczema, which might point to an increased tendency for a hyperergic response to a variety of antigens.

The basic problems prompting immunological research in ischaemic heart disease concern the pathogenetic significance of autoantibodies occurring in the process of myocardial infarction. The question whether the immunological mechanisms associated with the necrosis of the heart are involved in the development of the clinical symptoms arouses lively interest (Kaplan, 1958; Ehrenfeld, Gery, and Davies, 1961; Van de Geld, 1964; Faivre *et al.*, 1967; Kuch, 1968).

Although the cytotoxic qualities of the autoantibodies under consideration remain hypothetical, there are nevertheless some arguments to support the hypothesis.

According to Grabar's (1957) concept, commissurotomy or necrosis of the heart muscle leads to changes in the special structure of protein, thus endowing it with autoantigenic qualities. Combination of the resulting antibodies with intracellular structures would impair the functions of the latter and could be conducive to untoward effusion-generating reactions.

We note a relationship between the immunological phenomena and the clinical course in cases of Dressler's post-infarction syndrome. In these cases, stormy manifestations of the disease usually coincide with an increase in the titre of the antibodies in the serum. And, conversely, corticotherapy is followed by a rapid and effective clinical improvement and also reduction of the titre of antibodies.

The case of patient J.K. with pemphigus, in whom accidental detection of anti-heart antibodies lead to the diagnosis of myocardial infarction, throws a certain light on the possible involvement of these antibodies in the pathogenesis of the post-myocardial infarction syndrome (Chorzelski and Kuch, 1969). In this patient, the titre of the antibodies was as high as 1:320, which is a level we have seen only in Dressler's syndrome, though the actual syndrome did not develop.

It is possible that corticosteroids, administered in this case for pemphigus some time before myocardial infarction was diagnosed, caused Dressler's syndrome to run an abortive course – that is, inhibited the expected effects of the high titre of antibodies. This interpretation is supported by the observation that patients suffering from Dressler's syndrome respond promptly to treatment with corticosteroids.

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