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Humoral rejection after heart transplantation: reliability of intramyocardial electrogram recordings (IMEG) and myocardial biopsy

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Abstract In recent years, as the importance of humoral-mediated rejection has increasingly become recognized, the fact that endomyocardial biopsies (BX) evaluated according to the criteria of the International Society for Heart and Lung Transplantation often produce false-negative results has become a matter of concern. To evaluate the reliability of measuring intramyocardial ECG amplitude (IMEG) and immunofluorescence evaluation (FITC-labeled anti-IgG/IgM staining) of endomyocardial biopsies (IFM), heterotopic neck-heart transplantation (HTX) was performed on eight beagles previously sensitized through skin transplantations. After HTX, IMEG, echo, and donor-specific antibodies in serum (IgG, IgM) were determined daily and myocardial biopsies (IFM, BX) were performed once every 2 days. Accelerated (humoral) rejection occurred on the 5th (4th–5th) postoperative day and sensitivity of IMEG, IFM, and BX was 100 %, 75 %, and 12.5 %, respectively. In each case rejection was recognized so early that it was possible to initiate therapy with "restitutio ad integrum". Our results show that, as opposed to endomyocardial biopsy (IFM, BX), IMEG diagnosis detected humoral-mediated rejection early and with high reliability.

Key words Intramyocardial ECG · IMEG, heart transplantation · Humoral rejection, heart transplantation · Rejection, heart transplantation, humoral

Introduction

Humoral rejection after heart transplantation has a markedly poorer prognosis than cellular rejection [4, 5, 8, 10, 12]. A fundamental reason for this is that such episodes are not detected by the evaluation of endomyocardial biopsies according to International Society for Heart and Lung Transplantation (ISHLT) guidelines due to the absence of lymphocytic infiltration [1, 2], thus leading to a delay in urgently needed rejection therapy. Although Hammond et al. have suggested guidelines for immunofluorescent microscopic proof of humoral rejection [8, 9], the value of immunofluorescence after heart transplantation is not without controversy [3]. Furthermore, as it is an invasive procedure, immunofluorescence cannot be performed daily, a disadvantage that is particularly aggravated by the extremely quick development of humoral rejection.

Intramyocardial ECG (IMEG) is a noninvasive procedure which, together with echocardiography, has replaced endomyocardial biopsy for routine diagnostics at our facility [11, 15]. We previously showed that not only lymphocytic infiltration but also the electrophysiological changes exhibited a focal distribution during acute rejection episodes [6]. Furthermore, when several IMEG electrodes were used, the lines exhibiting the greatest sensitivity were those within a focus of rejection (ISHLT grade 3). This presented the prospect that myocytolysis and/or lymphocytic infiltration

were the causative factors associated with the decrease in voltage. For this reason, it was feared that IMEG diagnosis would also fail to detect humoral rejection. The aim of this study, therefore, was to evaluate the reliability of intramyocardial ECG monitoring (IMEG) during episodes of humoral-mediated rejection.

Material and methods

Protocol and experimental model

In order to investigate humoral-mediated rejection, sixteen beagles underwent heterotopic neck-heart transplantation. Eight dogs (experimental group) were sensitized through skin transplantations as described by Rapaport et al. [13]. After 14 days, the animals received a second skin transplantation from the same donor. To prevent hyperacute rejection, the heart transplantation was performed on the 28th day after the first skin transplantation if donor-specific antibodies had already disappeared from circulation. The heart transplantation technique used has been thoroughly described previously [6]. Epimyocardial puncture electrodes (Vascumed, Weil a. Rhein) were inserted in the right and left ventricles, as well as in the cardiac apex, of the allografts. During heart transplantation, an additional pacer electrode was placed in the native heart of four of the dogs. All of the electrode lines exited percutaneously from the nape. After the heart transplantation, the beginning and subsequent course of rejection episodes was monitored by IMEG measurements, echocardiography, serial myocardial biopsies, and follow-up of donor-specific antibodies in serum. The first episode of rejection was treated; after the onset of the next episode, the experiment was discontinued and an autopsy performed. A group of eight dogs that had undergone heterotopic neck-heart transplantation without previous sensitization served as controls. These dogs received the same treatment as the experimental group. All of the animals were handled in accordance with the principles of laboratory animal care (NIH publication No. 86–23, revised 1985) as well as with the German law regarding animal care.

Immunosuppression

Intraoperatively, as well as on the first 2 postoperative days, the dogs received 250 mg methylprednisolone. A maintenance dose of prednisolone (0.3 mg/kg) was subsequently administered. Cyclosporin A was initially administered daily (2×10 mg/kg) after heart transplantation and was then adjusted to a whole blood level of 400–600 ng/ml. The azathioprine dosage was 2 mg/kg. In the group of sensitized dogs, therapy for the first rejection episode was accomplished with cortisone boli, apheresis, and cyclophosphamide. Rejection therapy was not necessary during the first 10 days after transplantation in the group of non-sensitized dogs.

Myocardial biopsy

In the experimental group, biopsies were performed on day 3 (BX1), prior to the start of antirejection therapy on day 4 or 5 (BX2), at the end of the rejection therapy (BX3) on day 7 or 8, and at the beginning of the second rejection episode on day 9 or 10 (BX4; Table 1). In the control group, biopsies were performed at the same time points. Myocardial biopsy was performed with the dogs under short-term anesthesia, whereby the skin over the allograft was reopened and a transmural punch cylinder removed. Lymphocytic infiltration was evaluated according to the ISHLT classification [2]. In addition, the extent of myocardial edema was graded as: 0 (no edema), 1 + (mild edema), 2 + (moderate edema), or 3 + (severe edema). Lastly, the specimens were examined via immunofluorescent microscopy and classified according to the guidelines suggested by Hammond et al. [9]. For this purpose, antibodies directed against dog IgG and IgM (Binding Site, Birmingham, UK) were used.

Intramyocardial ECG measurement (IMEG)

For each measurement, the maximum amplitude of 30–40 consecutive QRS complexes was averaged. This average was considered a relative value corresponding to the initial voltage measured at transplantation (= 100 %). Three bipolar leads were recorded from the three electrodes during each measuring period. Based upon previous clinical and experimental experience, a decrease in voltage of 10 % or more was considered an indication of rejection. Measurements were conducted daily between 8:00 a.m and 10:00 a. m. on both the transplanted and native hearts.

Echocardiography

During each IMEG measurement, echocardiography was used to determine (end-diastolic) left ventricular wall thickness and maximal diastolic relaxation velocity. The values of both parameters were also considered relative values corresponding to the initial value. As described previously, the initial value (100 %) was registered on the third postoperative day, after the reduction of the edema caused by ischemia/reperfusion [7]. In order to minimize interobserver variability, all measurements were made by the same examiner. Using the M-mode, all measurements were made at the free posterior wall between papillary muscle and mitral valve at a site marked during transplantation in order to minimize day-to-day variability.

Analysis of donor-specific serum antibodies

This measurement was only performed on sensitized dogs. Serum specimens of the recipient animals were taken weekly after the skin transplantations and daily after heart transplantation, and the frozen at –20 °C. The spleens of the donor animals were also removed when the hearts were procured. After the experiment was terminated, the serum specimens of the recipients were incubated with the splenocytes of the donors. The donor-specific antibodies bound to the splenocytes were marked with FITC-marked sheep antibodies (Binding Site, Birmingham, UK) directed against dog IgG and IgM, respectively, and detected by cytofluorometry (FACSscan, Becton-Dickinson, Heidelberg).

Data analysis

The values of intramyocardial voltage (IMEG), left ventricular wall thickness, and maximal diastolic relaxation velocity were considered relative values since the interindividual variation of the absolute values lie within the range of the changes expected during rejection. The median value and range were calculated.

Table 1 Results of myocardial biopsies in sensitized (*S*) and nonsensitized (*NS*) dogs (*BX1* exclusion of rejection (3rd day), *BX2* proof of first rejection immediately before therapy was begun (4th–5th day), *BX3* monitoring after the end of therapy (7th–8th day), *BX4* proof of second rejection, *ISHLT* light microscopic evaluation according to ISHLT classification [2], *edema* light microscopic evaluation of the myocardial edema and classification according to a scale of −3 + (no edema, mild, moderate, severe), *IgG* and *IgM* immunofluorescent microscopic evaluation of IgG and IgM according to the classification suggested by Hammond et al. [3]

Group	Dog	Method	BX 1	BX 2	BX 3	BX 4
	I	ISHLT	0	1a	0	3a
S	I	edema	0	3 +	0	3 +
	I	IgG	0	2 +	1 +	1 +
	I	IgM	0	0	1 +	0
	II	ISHLT	0	2	1a	3b
S	II	edema	0	3 +	0	2 +
	II	IgG	1 +	3 +	1 +	1 +
	II	IgM	0	1 +	0	1 +
	III	ISHLT	0	0	1a	3a
S	III	edema	0	2 +	0	1 +
	III	IgG	0	3 +	2 +	2 +
	III	IgM	1 +	0	1 +	1 +
	IV	ISHLT	0	0	3a	3a
S	IV	edema	0	2 +	0	1 +
	IV	IgG	0	2 +	2 +	2 +
	IV	IgM	1 +	0	1 +	1 +
	V	ISHLT	0	1a	1a	3b
S	V	edema	0	3 +	1 +	3 +
	V	IgG	0	2 +	2 +	2 +
	V	IgM	0	1 +	0	2 +
	VI	ISHLT	0	0	0	3a
S	VI	edema	0	2 +	0	2 +
	VI	IgG	0	3 +	1 +	1 +
	VI	IgM	0	1 +	0	0
	VII	ISHLT	0	0	0	2
S	VII	edema	0	2 +	1 +	1 +
	VII	IgG	1 +	1 +	1 +	1 +
	VII	IgM	0	0	1 +	1 +
	VIII	ISHLT	0	0	3a	3b
S	VIII	edema	0	3 +	0	2 +
	VIII	IgG	0	2 +	2 +	1 +
	VIII	IgM	0	0	1 +	1 +
	1	ISHLT	0	1a	0	0
NS	1	edema	0	0	0	0
	1	IgG	1 +	0	0	0
	1	IgM	0	0	0	0
	2	ISHLT	0	0	0	1a
NS	2	edema	0	0	0	0
	2	IgG	0	0	0	0
	2	IgM	0	1 +	0	0
	3	ISHLT	0	0	1a	0
NS	3	edema	0	0	0	0
	3	IgG	1 +	0	0	0
	3	IgM	0	0	0	0
	4	ISHLT	0	0	0	0
NS	4	edema	0	0	0	0
	4	IgG	0	1 +	0	0
	4	IgM	0	0	0	0
	5	ISHLT	0	0	0	0
NS	5	edema	1 +	0	0	0
	5	IgG	1 +	0	0	1 +
	5	IgM	0	0	0	0
	6	ISHLT	0	0	0	0
NS	6	edema	0	1 +	0	0
	6	IgG	0	0	0	1 +
	6	IgM	0	0	0	0
	7	ISHLT	1a	0	0	0
NS	7	edema	0	0	0	0
	7	IgG	0	0	0	0
	7	IgM	0	0	0	0
	8	ISHLT	0	0	0	0
NS	8	edema	0	0	0	0
	8	IgG	0	0	0	0
	8	IgM	1 +	0	0	0

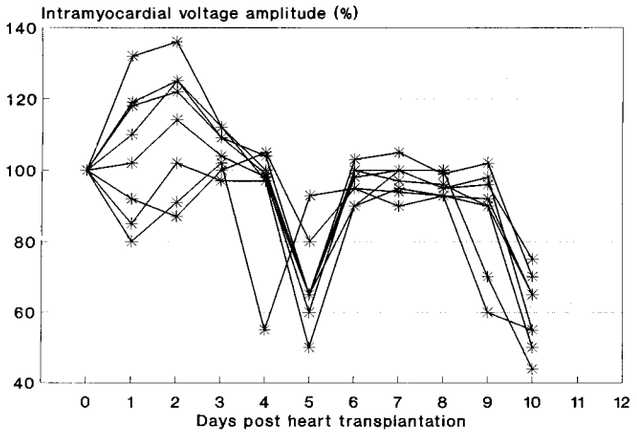

Fig. 1 Course of intramyocardial voltage amplitude (IMEG) from the allografts of eight sensitized recipients. Rejection occurring on the 5th (4th–5th) as well as 10th (9th–10th) days are recognizable by the marked decrease in the IMEG. The immediate increase in IMEG to its initial value on the 6th day documents the successful therapy of the first episode of rejection. The readings from only one of the three leads from each allograft are shown

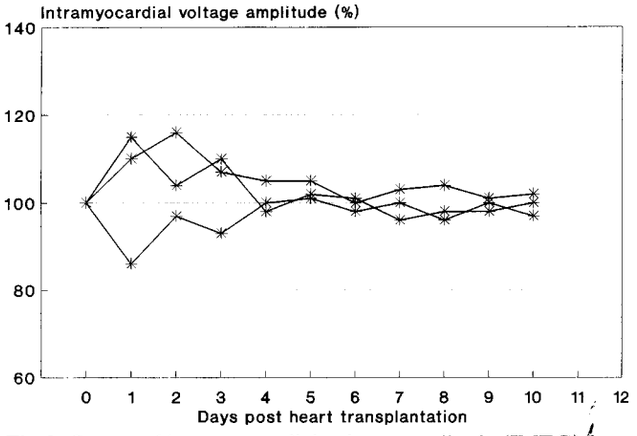

Fig. 2 Course of intramyocardial voltage amplitude (IMEG) from the allograft of a nonsensitized recipient. The variation in the course of the voltage amplitude of the three leads from the allograft, which occurred during the 4- to 5-day stabilization phase, indicates that the electrical activity occurring during this time frame was not homogeneously distributed over the myocardium. In the absence of rejection, the voltage remained stable during the further course after reaching the plateau phase. The readings from all three leads from each allograft are shown

Results

The success of sensitization in each of the eight dogs that had undergone skin transplantation was evident from the marked increase in donor-specific antibodies (IgG) observed a week after each skin transplantation. Furthermore, the second set of transplants was rejected after only 6 days (range 6–7 days) while the first set survived 10 days (range 8–13 days).

In the group of sensitized dogs, neither biopsy nor noninvasive parameters exhibited any indication of rejection on the 3rd day after heart transplantation. On the 5th (4th–5th) postoperative day, all of the transplants were severely rejected within 1 day due to accelerated rejection. Biopsies revealed moderate (2 +) to severe (3 +) myocardial edema as well as a moderate (2 +) to severe (3 +) marking under immunofluorescence (Table 1). Lymphocytic infiltration with isolated myocytolysis was observed in only one case. In every case there was a significant decrease in IMEG of 38 % (20 %–50 %; Fig. 1), an increase in left ventricular wall thickness of 18 % (14 %–24 %), and a decrease in maximal diastolic relaxation time of 25 % (20 %–30 %; Table 2). Furthermore, all of the rejection episodes were accompanied by a high serum titer of donor-specific antibodies.

A comparison of the three leads from a single allograft showed that, although there were distinct differences during the first days, all of the leads behaved identically, both before and during humoral rejection once voltage had stabilized. Therefore, only measurements between the right and apical electrodes of each allograft are given (Figs. 2, 3).

Table 2 Course of the noninvasive parameters during humoral-mediated rejection. All values are relative (*During rejection* difference between uncompromised cardiac function on the 3rd day and maximal functional loss until the initiation of therapy on the 5th day, *After therapy* difference between cardiac function at the beginning and end of the 3-day therapy)

Animal no.	Intramyocardial voltage amplitude		Left ventricular wall thickness		Maximal diastolic relaxation velocity	
	During rejection	After therapy	During rejection	After therapy	During rejection	After therapy
I	– 45 %	+ 40 %	+ 20 %	– 20 %	– 25 %	+ 25 %
II	– 40 %	+ 40 %	+ 21 %	– 20 %	– 30 %	+ 30 %
III	– 50 %	+ 45 %	+ 17 %	– 19 %	– 25 %	+ 20 %
IV	– 35 %	+ 35 %	+ 18 %	– 19 %	– 25 %	+ 25 %
V	– 35 %	+ 35 %	+ 18 %	– 17 %	– 20 %	+ 25 %
VI	– 20 %	+ 15 %	+ 14 %	– 15 %	– 25 %	+ 25 %
VII	– 35 %	+ 40 %	+ 18 %	– 18 %	– 20 %	+ 20 %
VIII	– 45 %	+ 45 %	+ 24 %	– 25 %	– 30 %	+ 30 %
Median	– 38 %	+ 40 %	+ 18 %	– 19 %	– 25 %	+ 25 %

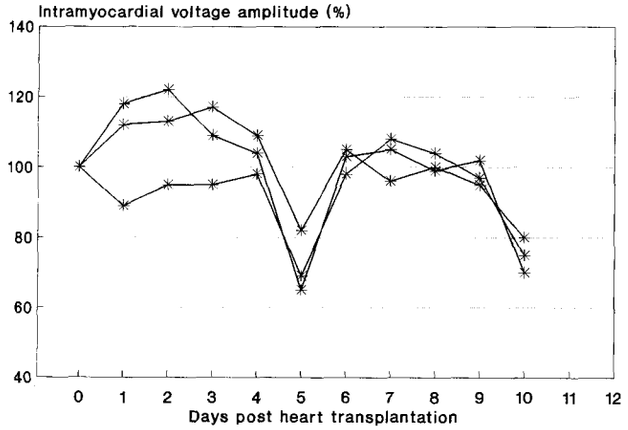

Fig. 3 Course of the intramyocardial voltage amplitude (IMEG) from the allograft of a sensitized recipient. The variation in the course of the voltage amplitude of the three leads from the allograft, which occurred during the initial stabilization phase, indicates that the disturbances in electrical activity occuring at this time were not homogeneously distributed over the myocardium. Rejection, which occurred on the 5th and 10th days, is recognizable by the marked decrease in the IMEG. The success of the therapy for the first rejection is documented by the immediate increase in IMEG to its initial value on the 6th day. The readings from all three leads from each allograft are shown

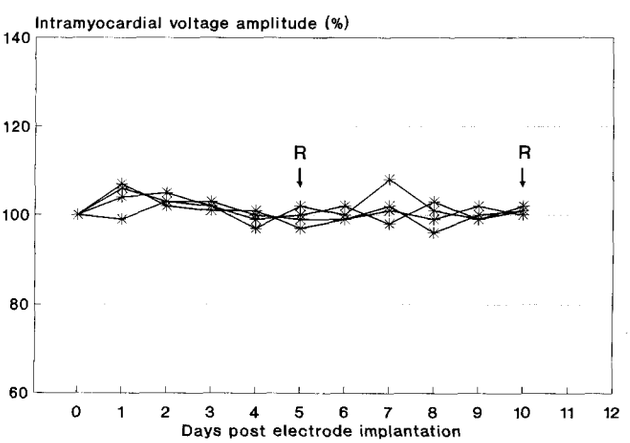

Fig. 4 Course of the intramyocardial voltage amplitude from the native hearts of four sensitized recipients. The voltage remains stable from the 1st day onward and, moreover, does not change despite humoral rejection (*R*) of the allograft implanted in the neck

Under rejection therapy with apheresis, cortisone boli, and cyclophosphamide, an almost complete disappearance of myocardial edema to grade 0/1 + was observed in all eight cases during the first 24 h. Left ventricular wall thickness and maximal diastolic relaxation time concurrently returned to their initial levels (Table 2). In a case of mixed rejection (cellular and humoral), the lymphocytic infiltration decreased from ISHLT

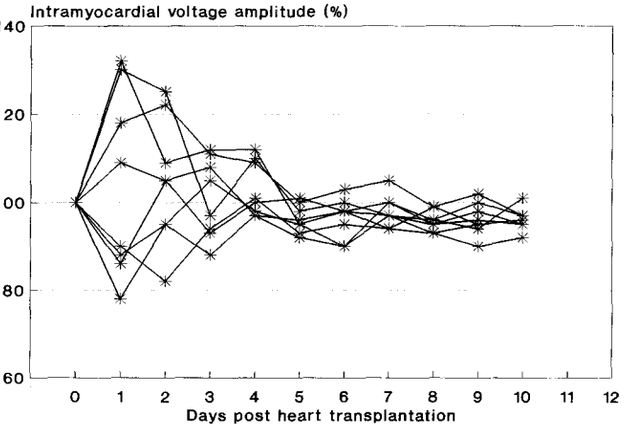

Fig. 5 Course of the intramyocardial voltage amplitude (IMEG) from the allografts of eight nonsensitized recipients. After a stabilization phase of 4–5 days, all of the voltage amplitudes reached a plateau which, in the absence of rejection, remained stable during the further course. The readings from only one of the three leads of each allograft are shown

grade 3A to 1B. Although rejection therapy was able to remove donor-specific antibodies from serum, immunofluorescent microscopy findings remained unchanged (Hammond Classification 1 + – 3 + ; Table 1).

The intramyocardial voltage (IMEG) in the native hearts of the sensitized recipients did not change during the entire 10 days (Fig. 4).

Neither the biopsy nor the noninvasive findings from the nonsensitized dogs indicated rejection during the first 10 postoperative days (Table 1). Nevertheless, distinct variations in intramyocardial voltage occurred during the first 4–5 postoperative days (Fig. 5). Only later during the course of the experiment did the values plateau.

Discussion

In this study, a model of accelerated rejection after previous sensitization through skin transplantation was chosen. As anticipated, after only 5 days, a very aggressive course of almost exclusively humoral-mediated rejection set in, such that, with the exception of edema, light microscopy exhibited no manifestation of rejection. In only one of the eight cases was relevant lymphocytic infiltration was observed in association with the humoral component in the sense of "mixed rejection". On the other hand, in the biopsy specimen (BX2) from sensitized dogs, FITC-labeled anti-IgG showed a moderate to severe staining (grade 2 + /3 +) whereas, in accordance with the uneventful clinical courses in the control group, no or only mild myocardial staining (grade 0/1 +) was found in the biopsies (Table 1).

After heart transplantation the amplitude of intramyocardial ECG (IMEG) required 4–5 days in the absence of rejection to stabilize at a plateau (Fig. 5). If one compares the different leads from the same allograft during this early phase, one finds that voltage behavior is not homogeneous, whereas such variances are no longer evident once a plateau has been achieved after the 4th or 5th postoperative day (Figs. 2, 3). Since the IMEG of the native heart remained stable from the 1st day, electrode implantation, narcosis for transplantation, and the initially high cortisone doses could all be excluded as causes of the initial voltage deviations (Fig. 4). An immunological cause at this early stage also seems improbable, particularly since at this phase the measurements in both the sensitized and non-sensitized recipients did not differ from one another (Figs. 1, 5). Obviously, the disturbances that occur in the electrical activity of the myocardium during the first days after cardioplegic arrest are not homogeneously distributed.

Since the sensitization used in this study led to the very early onset of rejection, no plateau could be distinguished in the course of the IMEG at that time (Fig. 1). Nevertheless, a comparison of these curves with the spontaneous course in Fig. 5 left no doubt that the sudden decrease in voltage was caused by rejection. Remarkably, although only one case involving lymphocytic infiltration was observed, a significant decrease in voltage of greater than 10 % occurred in all cases. The sensitivity of IMEG for humoral rejection was, therefore, 100 %, while for myocardial biopsy it was unacceptably low (12.5 %) when evaluated according to the criteria of the ISHLT. However, when the myocardial biopsy was examined by immunofluorescence, sensitivity was 75 % (6/8) if no or only mild anti-IgG staining (grade 0 or 1 +) was defined as inconspicuous and moderate or severe staining (grade 2 + or 3 +) was regarded as an indicator of humoral rejection.

In this present study, the decrease in voltage at the time of diagnosis was already 38 % (20 %–50 %), or four times greater than necessary for diagnosing rejection, despite daily measurements. Conducting measurements at a specific time at night while patients sleep has proven to be clinically useful since intramyocardial voltage amplitude is influenced not only by great variability during the day, but also by a range of other factors (stress, etc.) [14]. This explains why measuring IMEG several times throughout the day can lead to difficulties in interpreting the data. It must be noted, however, that in every case, daily IMEG measurements detected rejection so early that successful therapy with restitutio ad integrum was possible (Table 2).

Although IMEG readings, left ventricular wall thickness, maximal diastolic relaxation time, and light microscopic findings completely recovered during successful therapy and donor-specific antibodies in serum were greatly eliminated, immunofluorescent microscopy continued to detect bound antibodies in the allograft (Table 1). IMEG is thus also better suited for the clinical monitoring of therapeutic success than repeated myocardial biopsies with evaluation by immunofluorescent microscopy.

As the authors have previously shown, the decrease in voltage caused by rejection begins focally and spreads throughout the entire myocardium during the course of persistent rejection [6]. A comparison between voltage amplitude and histological findings from the area around the affected electrodes indicated that lymphocytic infiltration, which initially occurs focally, is obviously also the initial site of electrophysiological change. On the other hand, myocytolysis corresponding to the R-decrease associated with cardiac infarction has often been used to explain the decrease in voltage observed during rejection. For this very reason, IMEG had previously been suspected of detecting rejection too late. However, in this study, the decrease in voltage observed during episodes of humoral rejection showed that the underlying electrophysiological changes obviously were not necessarily associated with myocytolysis or lymphocytic infiltration. Furthermore, this decrease in voltage proved to be quickly reversible, thus indicating functional impairment, rather than structural damage, of the myocardium. This may be attributed to the effect of inflammatory mediators, which also play a role in episodes of humoral rejection.

The fact that the rejection episodes observed in this study exhibited no significant differences in the registrations of the different leads from the same allograft initially seems to support the notion that the humoral course does not originate focally, but globally, affecting the entire myocardium equally from the outset (Fig. 3). Nevertheless, the authors have observed patients who had experienced humoral rejection in which only one of two leads indicated a decrease in voltage. However, these episodes continued over several days and, therefore, the explanation for the identical behavior of the different leads of an allograft may have been associated with the subsequent rapid development of rejection.

The results of this present study indicate that measuring intramyocardial voltage amplitude (IMEG) detects humoral-mediated rejection early and with high reliability, therefore closing the diagnostic gap of endomyocardial biopsy. Furthermore, measuring the IMEG is suitable for monitoring rejection therapy. In contrast, immunofluorescent microscopic evaluation of myocardial biopsies is less reliable, as its sensitivity is lower (75 % vs 100 %). As an invasive method, it is not feasible on a daily basis, which is a profound disadvantage with respect to the rapid course of humoral rejection episodes. Furthermore, the method is not suitable for monitoring rejection therapy, since the myocardial staining remains unchanged despite successful therapy.

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