Lack of effect of rapamycin in anti-CCP antibody production in a rheumatoid arthritis kidney allograft recipient

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ABSTRACT

Autoimmune diseases may lead to end-stage renal disease and, as a consequence, kidney transplantation. Classical immunosuppressive drugs, such as cyclosporine or corticosteroids, are well-established therapies for both transplantation and autoimmune diseases. Rapamycin is a new immunosuppressant useful for allograft transplantation and with a promising future for autoimmune diseases, although it has not been extensively studied in humans. Here the case of a patient diagnosed with rheumatoid arthritis (RA) who received a renal allograft is reported. She was started on prednisolone, azathioprine and cyclosporine immunosuppression and changed to rapamycin instead of cyclosporine 4 years after transplantation, because of chronic allograft nephropathy. At present, the patient has a functioning graft. However, the arthritis symptoms reappeared after the change in immunosuppressant. Titers of RA-specific anti-cyclic citrullinated peptides antibodies increased whereas rheumatoid factor titers decreased. This case report suggests that rapamycin used for kidney transplantation might have a different influence on the spectrum of RA autoantibodies.

Introduction

Autoantibodies directed to citrullinated proteins have been recently described as specific for the diagnosis of rheumatoid arthritis (RA) (1). New ELISA tests have been developed for the detection of anti-cyclic citrullinated peptides antibodies (anti-CCP Abs). Anti-CCPAbs are highly specific markers of RA (91-98%) and can be detected in approximately 70-80% of RA patients (2), in contrast to rheumatoid factor (RF) which has been demonstrated to be of low specificity (3). Whereas their specificity for the RA diagnosis is well established, their role as prognostic markers is still not clear (4, 5). Nor are the mechanisms involved in the production of anti-CCPAbs well elucidated (6). As in the case of other autoantibodies, it seems to be clear that a T-dependent B cell response after exposure to citrullinated peptides must occur for anti-CCP Abs production (6). Kidney transplantation is a clinical setting where alloimmune T-B interactions continuously occur that must be suppressed with immunosuppressive therapy. Rapamycin is an immunosuppressive agent with a new mechanism of action. It has been demonstrated to be an effective immunosuppressant in renal allograft recipients and allows the withdrawal of classical calcineurin inhibitors (7). The use of rapamycin in autoimmune diseases has been proposed and there are some experimental models where it has been effective (8), but it has not been proved in humans.

Here the case of a patient diagnosed with RA nine years before receiving a renal allograft is reported. The patient had positive titers of RF and anti-CCP Abs at transplantation. She was started on prednisolone, azathioprine and cyclosporine immunosuppression and changed to rapamycin instead of cyclosporine 4 years after transplantation. At present, the patient has a functioning graft. The evolution of Abs titers and its possible relationship with immunosuppression and the clinical course is discussed.

Case report

A 71-year old woman who had been diagnosed with RA (9) in 1990 developed end-stage renal failure of unknown etiology. Seven years after the RAdiagnosis she was on dialysis therapy for one year, and in 1998 she received a three-HLA antigen mismatched cadaveric renal allograft with a good peri-operative course. Over the following 4 years there was excellent allograft functioning on standard triple immunosuppression with cyclosporine, azathioprine and prednisolone.

In June 2002 the patient developed a chronic allograft nephropathy diagnosed by biopsy, and immunosuppressive therapy with rapamycin instead of cyclosporine was initiated. One year later, in August 2003, the patient was admitted into our unit because of fever of unknown origin. Finally, the patient was diagnosed with miliary tuberculosis and started on therapy with anti-tuberculosis therapy. Renal function remained stable at admission (serum cre-

atinine 2.5 mg/dl and urea 1.22 g/L). C reactive protein was increased (7.26 mg/dl) and RF was positive (52.4 mg/dl). To date, renal function remains stable with no need of hemodialysis replacement. From transplantation to change to rapamycin the arthritis was considered clinically quiescent. However, the patient referred increase in articular symptoms from then. Besides, the physical exam revealed arthritis in both wrists, and several metacarpophalangeal and proximal interphalangeal joints that improved with increase in the steroid dosage from 10 mg/day up to 15 mg/day. At that time, the X-ray demonstrated a characteristic structural damage with narrowing of the intra-articular space of the joints indicated above, together with erosions and subluxation in metacarpophalangeal and metatarsophalangeal joints.

Since anti-CCP Abs has been recently introduced as diagnostic tool for RA, we searched for the presence of those Abs measured by an ELISA(Eurodiagnostica, Arnhem, The Netherlands). The anti-CCPAbs titer at admission in August 2003 was 740 U/ml. After her informed consent, we measured the levels of anti-CCP Abs and RF in all the sera we had collected and kept at –80°C from the transplantation. Figure 1 shows that titers of both anti-CCP Abs and RF, that were positive before transplantation, decreased rapidly with the initial immunosuppressive regimen from then to the acute rejection episode, when titers of both autoantibodies became negative. Thereafter, titers of anti-CCP Abs and RF progressively increased, although the former reached the same or higher titers than at the perioperative time whereas the RF did not reach as high as then (Fig. 1).

Four years after transplantation, when rapamycin was introduced, RF decreased 3-fold and anti-CCP Abs remained at titers over those at the time of transplantation, with even a peak. The lack of response of anti-CCP Abs production with rapamycin that is observed in Figure 1 corresponded to the lack of correlation between anti-CCP Abs titers and rapamycin plasma levels ($r = 0.100$; $p = 0.873$). On the contrary, serum titers of RF kept an excellent negative correlation with rapamycin plasma concentration ($r = -0.900$; $p = 0.037$). No significant association was found between plasma cyclosporine levels and any autoantibody, although there was some tendency to be negatively associated with anti-CCPAbs ($r = -0.501$; $p = 0.057$). Interestingly, prednisolone dosage had a good inverse association with serum titers of anti-CCPAbs ($r = -0.529$; $p = 0.009$). Finally, erytrocyte sedimentation rate demonstrated a significant correlation with anti-CCPAbs titers ($r = 0.538$; $p = 0.014$), but not with RF levels ($r = 0.017$; $p = 0.942$).

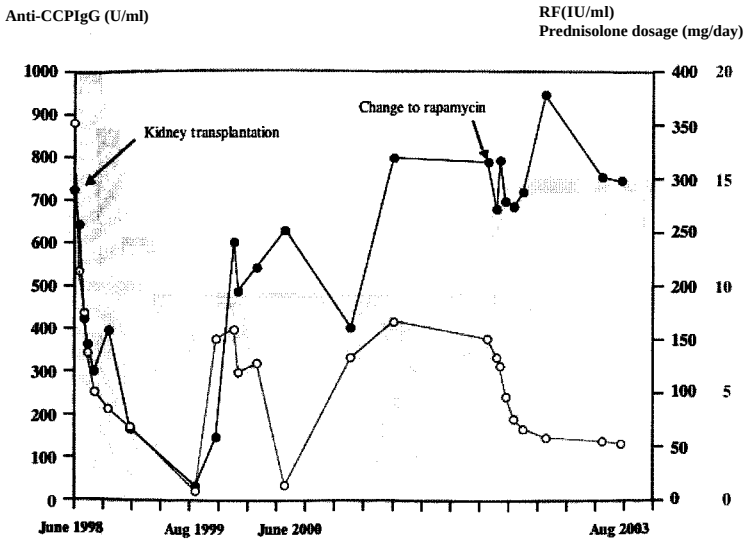

Fig. 1. Time course of levels of anti-CCPAbs (●) and RF (○) in the RAkidney allograft recipient. Grey shadow represents the prednisolone dosages that are indicated in grey numbers on the right axis. Follow-up goes from the day of renal transplantation (June 1998) up to the day of admission (August 2003). Main events are indicated with arrows.

Discussion

Anti-CCP Abs have been recently described as serologic markers of RAwith a limited sensitivity but a very high specificity (1-3). They are found in 70-80% RA but, more importantly, they are very helpful in diagnosing seronegative RA (1,2,10). Once their diagnostic value has been demonstrated, efforts are leaded to determine their prognostic utility (4, 5).

One of the most interesting findings from this case report is the failure of rapamycin to suppress the anti-CCP Abs production, at difference with the classical combination of azathioprine and cyclosporine. Rapamycin is effective in preventing allograft loss (7), as demonstrated in the patient reported. Some reports suggest their potential role in suppressing autoimmune responses in diseases such as RA (8). Moreover, rapamycin has been demonstrated to suppress MPO and ANCA titers in p-ANCA positive vasculitis before and after transplantation (11). These data suggest the capacity of this immunosuppressive drug to modulate autoimmune diseases (12).

Despite all of this evidence, our data indicate that rapamycin is not effective in suppressing the autoantibody response against citrullinated peptides in RA, although the RF, which is mostly IgM anti-IgG, was modulated. This may indicate that rapamycin, despite being a good suppressor of T cell proliferation (13), is not so effective as suppressor of T-B cell cooperation. The decrease in IgM RF could indicate that rapamycin may inhibit polyclonal B cell activation, or could also have to do with the lack of specificity of RF in RA. Two recent papers (14, 15) showed that blocking of TNFa with infliximab decreased titers of RF but not of anti-CCP Abs. Thus, anti-CCP Abs could act as a disease-specific marker for RA whereas RF could be related to disease activity.

The role of rapamycin in preventing RAprogression has not been objective-

ly evaluated. Here, rapamycin had little clinical effect, although titers of RF decreased but not of anti-CCP Abs. In contrast with it, cyclosporine seemed to manage better with RA, in terms of both serologic and symptomatic facts. Besides, plasma levels of cyclosporine showed an almost significant negative correlation with titers of anti-CCPAbs. Indeed, cyclosporine has been a well-established therapy in RA for many years (12). Furthermore, levels of cyclosporine were always above 200 ng/ml in the first year after transplantation, but decreased between 150 and 200 ng/ml when titers of anti-CCP began to rise. From Figure 1, prednisolone could be responsible for RF reduction following renal transplantation and after change to rapamycin. In turn, RF production was inhibited with prednisolone dosages higher than 10 mg/day, but not lower (Fig. 1). However, anti-CCPAbs did not follow the same time-course. Another explanation for the different course of titers of anti-CCPAbs and RF in our patient could reside in a differential regulation of the autoimmune response against both autoantigens as demonstrated in murine models (16) or suggested after TNFα blockade in humans (15).

The relationship between anti-CCPAbs levels and ESR was an interesting finding. Several reports have speculated about the possible effect of an inflammatory damage on the production of anti-CCP Abs in RA (6). The initial events that lead to the immune response against citrullinated proteins in our patient are unknown. However, the immune response against CCP would boost coinciding with subclinical inflammatory events occurring in the allograft, such as apoptosis. In this regard, citrullination has been proposed as one of a number of post-translational modifications of proteins occurring during apoptosis (17). By this mechanism, re-exposure to citrullinated peptides could be inducing the rising of anti-CCP Abs. In addition, a recent case report has been described of citrullinaemia in a hemodialysis patient, probably secondary to the inflammatory damage caused by both uremia and dialysis membranes bioincompatibility (18). Nonetheless, it is too speculative to suggest that citrullinaemia could be involved in the anti-CCP Abs production. Citrulline is a nonessential amino acid and a nitrogenous product in the small-intestine metabolism of glutamine. In turn, the plasma concentration of citrulline has been correlated with the small-intestine length (19). In our patient the influence of enterocyte mass on the plasma concentration of citrulline was excluded because there was no clinical/laboratory sign of malabsorption. Another possibility could be related with an impaired metabolism of citrulline since this amino acid is ultimately catabolized within the kidney to arginine (20). The impaired graft function in our patient might contribute to a delayed catabolism of citrulline and, subsequently, to increased citrullinaemia.

To our knowledge, we report the first documented case of RA in a kidney allograft patient in whom the evolution of the specific anti-CCP Abs has been monitored in relation with two different immunosuppressive regimens. We are aware of the uncommon of this clinical situation that cannot be completely translated to all RA, but should be taken into account when testing rapamycin in this autoimmune disease.

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