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Anti-integrin immunotherapy in rheumatoid arthritis: protective effect of anti-α4 antibody in adjuvant arthritis

Carmen Barbadillo[1], Alicia G-Arroyo[2], Clara Salas[3], Juan Mulero[1], Francisco Sánchez-Madrid[2], Jose L. Andreu[1]

[1] Servicio de Reumatología, Hospital Puerta de Hierro, c/San Martín de Porres 4, E-28035 Madrid, Spain
[2] Immunology Section, Hospital de la Princesa, Madrid, Spain
[3] Pathology Department, Hospital Puerta de Hierro, Madrid, Spain

Introduction

Rheumatoid arthritis (RA) is an inflammatory chronic disease that involves mainly the diarthrodial joints, although extra-articular manifestations such as rheumatoid nodules, interstitial lung disease, vasculitis and pleuresy frequently coexist. RA is a major health problem since RA is the most frequent organ-specific autoimmune disease with an incidence of between 2 and 4 in 10,000 every year when the adult population is considered, and a prevalence of 1% in the general population. Clinically, RA can present in several forms, the most frequent being an insidious onset with symptoms developing over the course of many years. The available treatments for RA are directed against different steps of the immune and chronic inflammatory response. Joint tenderness and swelling are partially controlled with analgesics and non-steroidal anti-inflammatory drugs. These drugs do not modify the outcome of disease, as they have a short-term effect. Disease modifying anti-rheumatic drugs (DMARD), such as parenteral gold, metothrexate and D-penicillamine, are used to improve the long-term outcome of RA. Treatment with DMARD cannot be used to control disease in a significant proportion of patients because of either the onset of adverse side effects or a lack of efficacy. In the majority of patients, RA progresses with time causing substantial morbility and mortality. The life expectancy of RA patients is reduced in a manner similar to that of patients with diabetes mellitus or with stage IV Hodgkin disease [40], and RA is the direct cause of death in 21% of RA patients [46]. At present it is clear that there is no treatment that can control RA in a way that actually modifies the long-term outcome for patients. Therefore, there is a need for the development of new therapeutic approaches in RA.

Monoclonal antibody (mAb) therapy for non-malignant disease was first introduced in organ transplantation. Injection of anti-CD3 OKT3 mAb led to a significant reversal of kidney transplant rejection [37]. During the last several years, a number of

Correspondence to: J.L. Andreu

trials using mAb therapy in RA have been performed. Most of these mAb are directed against molecules that could be relevant in the development of RA, the majority of which are localized in the membrane of CD4 T cells [21, 23, 26, 27, 35, 47, 52, 57], althought several studies have been published showing the therapeutic effects of mAb against interleukin (IL)-6 [58] and tumor necrosis factor-α (TNF-α) [13]. Although most of these trials have been conducted in a non-blinded, non-controlled fashion, the results suggest that mAb therapy for RA is safe and could provide significant and prolonged relief for patients with RA.

Relevance of VLA-4/VCAM-1 interaction in RA

The rheumatoid synovial membrane is characterized by the infiltration of large numbers of T lymphocytes into the hyperplastic inflammatory tissue. Many observations suggest that activated T cells initiate the inflammatory events in rheumatoid synovium that ultimately ead to tissue destruction [15, 25, 28]. In fact, arthritis can be induced by the injection of T cells in the absence of additional antigens, as demonstrated in experimental models [22]. It s clear that the presence of specific arthritogenic T cell clones and the migration into the synovial tissue is sufficient for the development of autoimmune arthritis in experimental models, with the recruitment not only of T cells but also granulocytes in synovial tissue. Therefore, since conventional therapies often do not lead to persistent clinical remission in RA, alternative therapeutic approaches have been used which reduce disease activity by depletion of T cells. These therapies include drainage of the thoracic duct [39], leukapheresis 14] and total lymph node irradiation [16], all of which frequently lead to a relevant clinical improvement.

Many investigations have focused on the further characterization of T cells in RA, both in the blood and intra-articular sites. These efforts have been hampered by the fact that no clearly defined antigen has been identified to date. Possible candidates for autoantigens are collagen type II, proteoglycans and chondrocyte membrane products [5]. However, it is not clear whether these antigens actually trigger the inflammatory processes or whether they merely represent attempts of the immune system to discard damaged cartilage tissue. In the absence of a definable antigen, strategies have been developed to suppress disease activity by the regulation of T cell function using mAb therapy directed against defined T cell epitopes. Many findings indicate that in RA intra-articular T cells have been activated in vivo. These observations include the presence of HLA class II antigens which are absent in normal T cells [4], the expression of CD2R [45], and the presence of other markers indicative of activation such as the CD69, a lymphocyte activation marker, and the integrin VLA-1 [20, 28, 29]. Overall, these observations suggest that T cells, selectively localized within joints, are activated to some extent in RA. However, additional findings on the small number of these cells and on their functional properties suggest that either the activation process has not been carried out ompletely or that cells, once activated, have rapidly reverted to an intermediate state of activation.

Activated T cells migrate to the synovial membrane by establishing interactions with the endothelial cells. Knowledge of the structure and function of cell adhesion molecules, which has progressively increased over the last few years, will allow a rational design of therapies both with mAb and synthetic peptides that have the capacity of blocking the physiological interactions of cell adhesion molecules with their ligands.

The integrin family is comprised by at least 22 different αβ heterodimers that mediate both cell-cell and cell-extracellular matrix interactions. Integrins are divided into different subfamilies according to the β subunit expression. The β1 integrins, also known as very late activation (VLA) antigens, include different receptors for extracellular matrix (ECM) (VLA-1 to VLA-9) with all having a common β1 chain and a different α chain [19, 48, 50]. VLA-4 (α4β1) posesses the capacity to mediate both cell-ECM as well as cell-cell interactions. Thus, VLA-4 interacts with VCAM-1 [12], a member of the superfamily of immunoglobulins, on activated endothelial cell and with fibronectin, a protein of the ECM [18, 54]. VLA-4 is expressed constitutively on T lymphocytes, B lymphocytes, natural killer cells, thymocytes, osinophils, basophils, mast cells and monocytes and its expression is up-regulated during lymphocyte activation and differentiation [17, 19, 41, 49, 56]. Long-term activation of T and B ells up-regulates the expression of VLA-4 [41, 49]. It is also well known that α4 integrin chain can associate with an alternative β chain, β7. The α4β7 integrin is present in a subset of T cells and mediates adhesion to VCAM-1, fibronectin, and MAdCAM [2, 50]. Furthermore, both α4β1 and α4β7 heterodimers can also function in homotypic lymphocyte aggregation [6, 43, 50]. Both α4β1 and α4β7 heterodimeric associations are expressed by T cells infiltrating the rheumatoid synovial membrane and its ligand, VCAM-1, is expressed not only by endothelial cells of the vessels of synovial membrane but also by fibroblasts and synoviocytes [10, 11, 29, 30, 33].

The emigration of lymphocytes from blood to synovial membrane requires their prior binding to endothelial cells. It is known that VCAM-1 is induced on endothelial cell surface in response to inflammatory agents [38, 51]. The participation of the VLA-4/VCAM-1 adhesion pathway has been implicated not only in rheumatoid arthritis but also in different chronic inflammatory diseases such as inflammatory bowel disease [36], glomerulonephritis [3], and others [31, 44]. T cell binding to activated endothelium involves mostly the LFA-1/ICAM-1 and VLA-4/VCAM-1 adhesion pathways [50]. In rheumatoid arthritis, the VLA-4-mediated binding capacity to VCAM-1 is increased in most synovial T cells [42]. Furthermore, an increased attachment of synovial T cells to fibronectin has also been observed [29, 34]. In conclusion, VLA-4 is up-regulated both in terms of expression and function by T lymphocytes of rheumatoid synovial membrane and it represents an interesting potential therapeutic target in RA. Blocking the binding of VLA-4 to its physiological ligands VCAM-1 and fibronectin could be of value in preventing the development of articular inflammation.

Anti-VLA-4 mAb treatment prevents adjuvant arthritis

The most widely used model of experimental arthritis for screening purposes is the disease produced by the subdermal injection of Freund's complete adjuvant into susceptible strains of rats [9]. The time of onset of adjuvant disease occurs from about day 10 onwards after the injection of the mycobacterial adjuvant, although reports vary from days 9 to 22. The disease has one-wave clinical course, starting at day 10, reaching a peak of joint inflammation from days 12 to 28, and the inflammatory process disappearing at days 35–40. This model shares several features with human RA. In both entities there is involvement of small and large joints with erosions of the adjacent bone, increase in the levels of acute-phase reactants and infiltration of synovial membrane by mononuclear cells. Furthermore, adjuvant arthritis is a T-cell-dependent process, with activated T cells infiltrating the synovial membrane [9]. It is

also possible to induce the disease passively by specific arthritogenic T cell clones in the absence of an antigenic insult [8]. In conclusion, adjuvant arthritis is an appropriate experimental model for investigating different therapeutic modalities of putative value in RA.

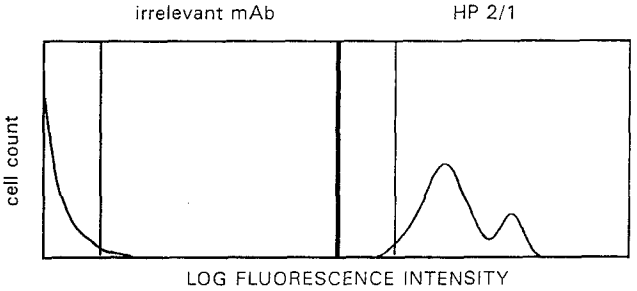

Fig. 1. Flow cytometry analysis of Lewis rat lymphocytes stained with monoclonal antibody (mAb) HP2/1. Cells were incubated with mAb HP2/1 at 50 $\mu g/10^6$ cells, followed by staining with a FITC anti-mouse antibody

The increasing knowledge about the role of adhesion molecules in the pathogenesis of different autoimmune and inflammatory diseases has led to the exploration of new therapeutic perspectives with reagents that can inhibit adhesion. Blocking of $\beta 2$ integrin functions by the use of mAb also suppress inflammation in a number of model systems [32]. Thus, anti-$\beta 2$ ameliorates antigen-induced chronic arthritis in rabbits [24]. Antibodies specific for the $\alpha 4$ ubunit of VLA-4 have been succesfully used in the treatment of different experimental inflammatory diseases [31]. Anti-$\alpha 4$ mAb reduce lymphocyte and neutrophil dermal infiltration and edema in contact cutaneous hypersensitivity responses [7]. Also, anti-$\alpha 4$ mAb inhibit the accumulation of eosinophils induced by intradermal chemoattractants, blocking posterior allergic response [55]. Furthermore, anti-$\alpha 4$ mAb also inhibit the development and progress of the induced experimental autoimmune encephalomyelitis by interfering with the leukocyte migration into the brain parenchyma [1, 59].

We have studied the effect of the HP2/1 mAb in modulating adjuvant rthritis in Lewis rats. HP2/1 is a mAb which recognizes the $\alpha 4$ subunit of VLA-4, blocking the binding of VLA-4 to its ligand VCAM-1. Since HP2/1 has no cytotoxic properties, its putative therapeutic effect resides in the ability to block the adhesion of VLA-4 to VCAM-1 and fibronectin. Although HP2/1 was generated in rats by immunization with human T cells, it also recognizes the $\alpha 4$ integrin from rats, as shown in Fig. 1. Therefore, it is a suitable reagent for the study of therapeutic effects on experimental models of arthritis in rats. A dose of 0.2 mg complete Freund's adjuvant was injected subdermically into the base of the tail of each rat. At day 10, 1 mg HP2/1 was injected intraperitoneally. As control groups, we used rats injected either with an isotype-matched irrelevant mAb, HP2/5 or the pan-leukocyte non-cytotoxic anti-CD45 (clone MRC OX-1, Serotec, Oxford, UK) mAb. The development of arthritis was followed daily by two observers in a blind fashion using a semi-quantitative score of arthritis. At day 35, animals were killed by CO_2 asphyxiation and the synovial membrane studied using hematoxylin and eosin staining. As shown in Fig. 2, only 10% of animals treated with HP2/1 developed arthritis, while 90% developed the disease among the groups treated with HP2/5 or OX-1. This difference was significant by Fisher's

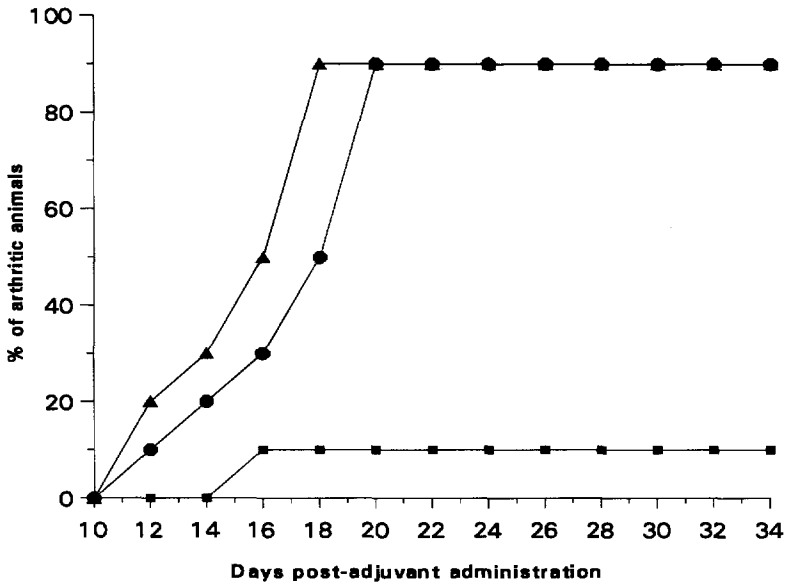

Fig. 2. Incidence of arthritis in female Lewis rats after administration of complete Freund's adjuvant and treatment with HP2/1 and control mAb (HP2/45 and OX-1); 10% of animals treated with HP2/1 developed arthritis in contrast with 90% of incidence among the control groups ($P = 0.001$; Fisher's exact test). -■- HP2/1; -●- HP2/5; -▲- OX-1

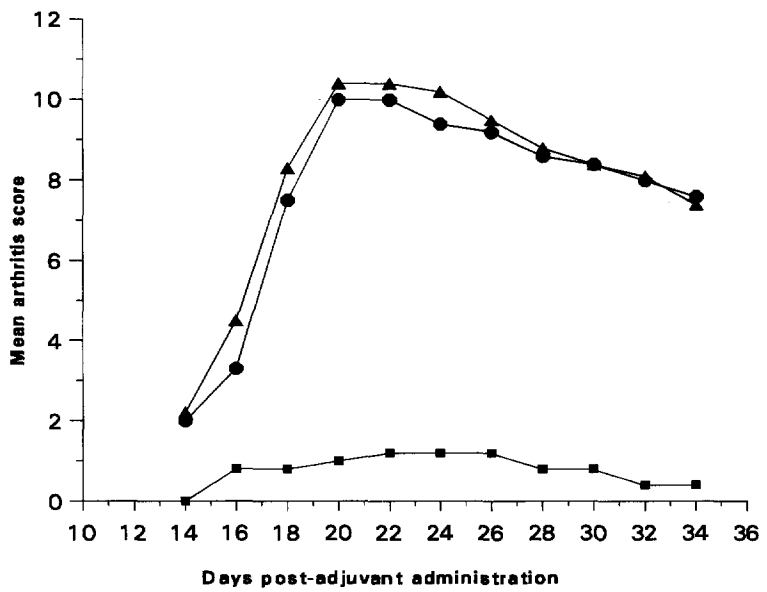

Fig. 3. Severity of arthritis in female Lewis rats after administration of complete Freund's adjuvant and treatment with HP2/1 and control mAb (HP2/5 and OX-1). The mean arthritic score in the group HP2/1 was siginificantly lower than in HP2/5 and OX-1 groups ($P < 0.005\%$; Mann-Whitney test, SE < 10%). -■- HP2/1; -●- HP2/5; -▲- OX-1

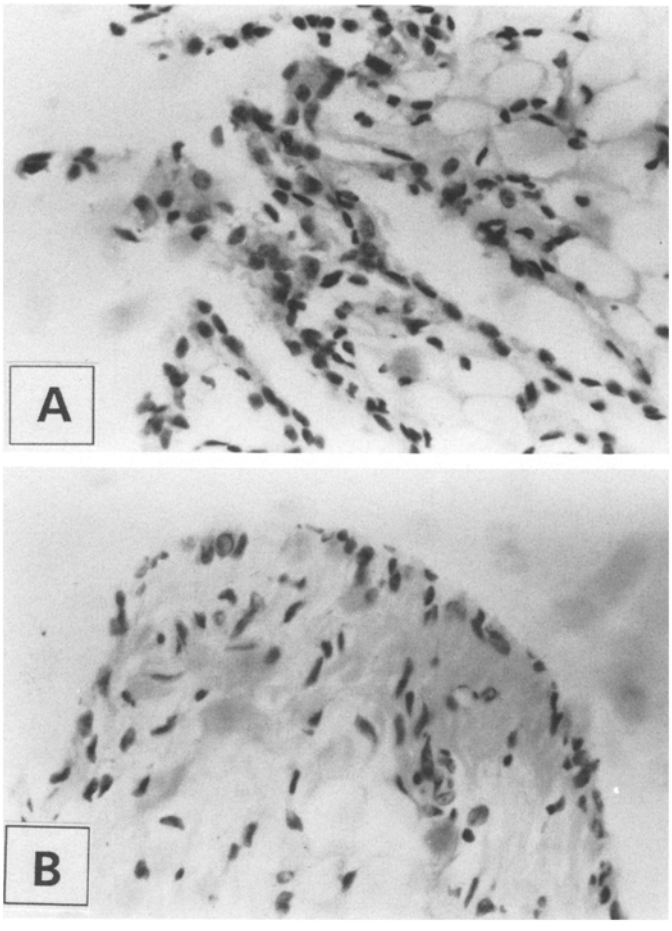

Fig. 4A,B. Histochemical analysis of synovial membranes derived from Lewis rats at day 35 after adjuvant administration. **A** Synovial membrane with the characteristic cell proliferation and papillary projections from a rat treated with control mAb OX-1. **B** Normal synovial membrane with a monolayer of synovial lining cells from a rat treated with HP2/1

exact test with a P value of 0.001. The semiquantitative score of arthritis in the different groups is shown in Fig. 3. Rats treated with HP2/1 did not develop arthritis, except one animal. Differences were again significant by Mann-Whitney test with a P value < 0.005. The pathological study of synovial membranes showed that, among the animals treated with HP2/1, only 10% had mononuclear infiltration and blood vessel proliferation in the synovial membranes. Synovial membranes from animals treated either with HP2/5 or OX-1 had mononuclear infiltration, edema and neovascularization. Figure 4 depicts representative examples from HP2/1-I and OX-1-treated animals. The characteristic proliferation of synovial lining cells and mononuclear infiltration in the synovial membrane from a rat treated with OX-1, was absent in the synovium derived from an HP2/1-treated animal. These results indicate that HP2/1 is effective in preventing the development of adjuvant arthritis in Lewis rats. The mechanism responsible for the favorable action of HP2/1 in adjuvant arthritis likely lies in

its ability to block VLA-4/VCAM-1 and VLA-4/fibronectin interactions as reported in other experimental models [1, 59]. In fact, the use of synthetic fibronectin peptides in arthritis that has been induced by bacterial cell wall products suppresses the disease by interrupting leukocyte adhesion [53]. Our data suggest that the treatment with antibodies specific for α4 integrins could be an interesting approach in the design of new immunotherapies for RA directed against cell adhesion molecules.

Conclusions

The current treatment of RA does not provide acceptable control of the inflammatory process due to either the onset of adverse effects or the lack of efficacy. RA is a T-cell mediated process, in which activated T cells must migrate through the blood vessels to reach the synovium. Anti-adhesion therapies designed to block the interaction of T cells with activated endothelial cells could be of great interest to control the disease. Flow cytometry analysis, immunohistochemical and functional studies cell adhesion in vitro have shown that the interaction between VLA-4 on T cells and VCAM-1 on endothelial cells could play a relevant role in the onset and perpetuation of RA. Here we show that the treatment with HP2/1, an mAb directed against α4 subunit of VLA-4, prevents adjuvant arthritis in Lewis rats. Data suggest that treatment with anti-α4 antibodies could be of value in RA.

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