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Unspecific, Targeted and Antigen-specific Immunomodulation in Collagen-induced Arthritis

Introduction

Several models of induced experimental arthritis which clinically resemble the human entity known as rheumatoid arthritis (RA) are used today. None of these models completely mimics RA and it is important to bear in mind that also the human disease described in textbooks of medicine may have several different disease entities in which the ethiopathogenetic mechanisms are shared only to some degree. If this is so, several pathogenic stimuli may induce the same clinical pattern of peripheral symmetrical joint inflammation, chronicity and joint destruction known as RA, in which rheumatoid factor (RF) production and HLA class II association are hallmarks.

Determination of the risk to develop RA in homozygous twins, shows that there is approximately a 15 % concordance rate to develop RA if one of the twins is diseased, i.e. 15 % of the disease susceptibility is conferred by inherited genes (1, 2). This also means that the risk to develop RA is to a great extent determined by external (environmental) factors. Because of the importance of environmental factors, undefined as yet in the human disease, it is of interest to find that bacterial products as well as mineral oils encompasses arthritogenicity in experimental models of arthritis. Furthermore, the combination of external (e.g. microbial adjuvants) and internal stimuli (e.g. collagen type II [CII]) is probably of importance for the chronicity of the experimental disease, since the models of adjuvant arthritis are usually not chronic (3), whereas collagen-induced arthritis (CIA) induced with homologous CII emulsified in adjuvant mineral oil has a chronic course (4, 5). Nevertheless, we must bear in mind that several pathways of pathogenicity have been hypothesised for the induction and the perpetuation of the clinical entity which we know as RA. This implicates that successful regimens for the treatment of RA need to be aimed at regulatory events which are in common for both external and internal disease-inducing and disease-perpetuating stimuli. An example of such a common pathway is the induction of proinflammatory cytokines such as TNF-α which can be induced both with adjuvants and antigens found in the external and internal milieu. An interesting in vitro observation that connects a cartilage component with the production of the TNF-α was recently published. In these experiments it was shown that TNF-α was produced in lymph node cell cultures derived from CII immunised rats only upon CII stimulation (6).

Inducible experimental models of arthritis can be initiated by immunisation of cartilage-derived antigens emulsified in an adjuvant or with adjuvants alone.[1] Examples of cartilage-derived molecules which induces joint inflammation are CII (4, 8 and reviewed in 9), cartilage oligomeric matrix protein (COMP) (Lorentzen et al, manuscript in preparation) and proteoglycan (PG) (10). Examples of adjuvants with arthritis-inducing capacities are mycobacteria dispersed in mineral oil (11) and mineral oils, e.g. Freund's incomplete adjuvant (FIA) (12) and pristane mineral oil (13-15). This means that experimental arthritis can be induced both with internal, e.g. cartilage-derived, antigens as well as with external stimuli, e.g. mineral oils. Further studies have demonstrated that the arthritis susceptibility is determined by MHC class II genes (3, 15) and is transferable by T cells both in adjuvant arthritis (16, 17) and CIA (18, 19). The uniting factor between these two models is the need of a Th1/proinflammatory adjuvant in order to set or tune the'arthritogenic' immune response. Of interest is the observation that rat strains which develop a more Th2 prone immune response after CII immunisation are resistant to disease induction, whereas other rat strains which displays a more Th1 prone immune response are susceptible to CIA (table 1 [20]).

Table 1.

Rat strains resistant to collagen-induced arthritis (Fisher and PVG) produce more IL-4 and less IFN-γ than susceptible the DA rat strain 7 days post immunisation (p.i.) with CII/FIA. Similar data were obatained at day 14 p.i. (data not shown). Cytokine mRNA was detected in an in situ hybridisation assay of lymph node cells from CII immunised rats. The figures indicate number of positive cells/10^5 lymph node cells (6).

Rat strain	IFN-γ	TNF-α	IL-4
DA	8	70	0
Fisher	0	20	0
PVG	0	18	7

This paper will focus on how immunomodulation which affects both specific and unspecific immunoregulation have been used in experimental CIA in rats and discuss the relevance of the present findings (many which have not yet been published) for future design of therapeutic trials in RA.

Two examples of experimental models of arthritis

Collagen-induced arthritis

Collagen type II is the major constituent of hyaline cartilage. Intradermal injection of native CII emulsified in Freund's incomplete adjuvant (FIA) induces arthritis in

[1] Models of joint inflammation which develops spontaneusly exist. Aging DBA/1 male mice devlop arthritis with a high incidence, somenthing which is coupled to their aggresive behavior. However, the pannus region and the inflammed synovial membrane contains no infiltrating T or B cells. This finding is discordant to the findings in human RA and CIA in which T cells and some B cells are found within the synovial membrane (7).

certain strains of rats and mice.[2] Immunisation with homologous collagen type II, i.e. rat CII in rats or mouse CII in mice, induces chronic joint inflammation (4, 5), whereas immunisation with heterologous collagen induces a self-limiting disease course (8). Disease susceptibility is conferred both by MHC class II genes (3, 22) and non-MHC genes (22, 23). In such arthritis susceptible strains both T cell and B cell responses to CII are present and of pathogenic importance.

Adjuvant arthritis and oil-induced arthritis (OIA)

It is of great conceptual importance that material without capacity to stimulate specific antigen-dependent immune responses is arthritogenic in certain inbred strains of rats and mice depending on their MHC and non-MHC genotype (3, 23).

During the late 1950s Pearson and co-workers found that heat-killed mycobacteria dispersed in mineral oil induced a non-chronic arthritis in Lewis rats (11). Subsequently it was demonstrated that the so called adjuvant arthritis model was dependent on a functional cellular immune response to mycobacteria and could be transferred from diseased to healthy irradiated recipient rats by T cell lines and clones of the CD4 lineage (24, 25). Interestingly, one of these disease-inducing clones was found to be cross-reactive to the human heat shock protein (hsp) 65 which is expressed in the synovial membrane in patient suffering from RA (26). This finding has led to further research of whether molecular mimicry exists between endogenously expressed antigenic determinants such as human hsp:s and microbial antigens such as bacterial hsp:s and whether such mimicry may be of pathogenic importance for the development of RA (27, 28).

New treatment strategies by using unspecific or targeted immunomodulation

The invention of the monoclonal antibody technique has opened new possibilities to design targeted immune intervention. In the 1980s monoclonals to T cell surface receptors as well as to immune response genes became available. These were soon used in experimental arthritis models. Treatment with antibodies specific for the CD4 and CD5 antigens but also to immune response gene antigens all affected the development of both CIA and adjuvant arthritis (29-31). However, effects on already developed disease were less dramatic. The use of antibodies to the CD4 receptor primarily detected on so called T helper cells has now been used in clinical trials of RA. So far clinical effects have been disappointing showing limited clinical effects on disease activity, but potentially serious adverse effects such as prolonged lymphopenia (32-34).

Many different approaches to down-regulate experimental arthritis have been tried. Many of these have also demonstrated measurable effects on arthritis development. However, it was not before the observation of a spontaneous development of erosive arthritis in transgenic mice which constituatively produced high levels of

[2] CIA is also inducible in nonhuman primates (21).

human TNF-α[3] and that TNF antibodies could prevent arthritis development in the TNF-α transgenic animals (35) that new a experimental understanding pawed the way to more successful clinicical investigations. In several other studies of rodent arthritis it was also demonstrated that anti-TNF treatment could ameliorate established disease as well (36-39). Today, the treatment with monoclonals to TNF-α or soluble TNF-α receptors have been and are used in clinical trials of RA (40, 41). The clinical success of this treatment has so far been an outstanding therapeutic achievement in clinical rheumatology. However, the dramatic clinical improvement seen in treated patients lasts only as long as the anti-TNF-α treatment is continued. When treatment is stopped or when an anti-idiotypic response to the injected antibodies develops, the disease relapses in many cases.

The further presentation and discussion of results from our laboratory in Stockholm and the laboratory of A. Tarkowsky in Gothenburg will make use of the experience of TNF-α treatment as an example of successful targeted immunomodulation. In table 2 examples are given of how antigen-specific, targeted and unspecific immunomodulation is used or may be used. The question I will address is whether unspecific or targeted immunomodulation may be combined with antigen-specific immunomodulation in order to enhance and prolong the anti-arthritic effect induced by one of these alone?

Table 2.

Immunomodulation	Examples of immuno-modulatory therapies	Examples from experimental and clinical trials
Unspecific	Corticosteroids, vitamin D_3, IVIG[1], irradiation, ABMT.[1]	Vitamin D_3 analogues. ABMT.
Targeted	Monoclonal antibodies to cell surface antigens and to cytokines. Soluble receptors antagonists.	Anti-CD4. Anti-TNF-α/rec. antagonist. Anti-IL1/rec. antagonist.
Specific	Co-administration of (disease-related) antigens. May be enhanced by selected adjuvants.	Oral collagen, MBP,[1] insulin ± cholera toxin B subunit. Bystander suppression. Alum adsorbed to collagen.

[1] Abbreviations: IVIG=Intravenous immunoglobulin; ABMT=autologous bone marrow transplantation; MBP=myelin basic protein

Effects of the Th2 inducing adjuvant alum combined with rat CII

CIA is associated with a proinflammatory (Th1) type of immune response and treatments with cytokines associated with a Th2 immune response are beneficial (6, 42-44).

[3] The only organ manifestetion these transgenic animals developed was joint inflammation. No evidence of inflammation was found in the spleen, the liver etc. (35-36)

Since agents with Th1-inducing properties such as FIA are necessary for disease induction, it was of interest to investigate whether an adjuvant with Th2-inducing properties affects CIA in a different way than does FIA. In a recently published study the arthritis development in DA rats after subcutaneous immunisation with the Th2 stimulatory adjuvant alum adsorbed to rat CII was studied (45). Such treatments suppressed disease development both prophylactically and therapeutically. The beneficial effect of alum/CII immunisation was associated with a changed pattern of production of subclasses of serum anti-rat CII antibodies, since the production of IgG1 antibodies was increases whereas the production of IgG2a and IgG2b anti- CII was decreased as compared to untreated rats or rats pre-treated with alum alone. In addition, alum/CII treated rats had a significantly higher expression of IL-4 mRNA than untreated rats in draining lymph nodes 7 days after CII immunisation. When the expression of mRNA for interferon-γ, IL-2 or IL-10 was measured no differences were found.

The demonstration that an increase in IL-4 as well as IgG1 autoantibody levels correlates with the beneficial effect of the alum/CII treatment indicates that this is due to a shift from a pro-inflammatory Th1 immune response to a Th2 immune response. Rats treated with alum or CII alone developed arthritis with a similar severity as non-vaccinated rats. In accordance, alum treatment prior to CII immunisation did not induce a shift towards IgG1 versus IgG2a/IgG2b anti-CII antibody production as was seen in the alum/CII treated rats.

Oral tolerance: Effects of cholera toxin B (CTB) conjugated to CII

To induce oral tolerance multiple administrations of large quantities of antigen is needed to cause deletion or anergy of antigen-specific T cells. If smaller amounts of antigen are used, Th2 immunity characterised by IL-4 and IL-10 production may be induced (46). However, when small quantities of disease-inducing antigens such as CII or myelin basic protein (MBP) have been fed the disease-protective effect has been only partial. This has been even more pronounced in the clinical trials so far completed in which patients with reumatoid arthritis and multiple sclerosis (MS). In MS no clinical improvement has so far been demonstrated (47). As for RA, the clinical effect was moderate at the treshhold of becoming statistically significant (D Hafler, personal communication). That such clinical trials have been undertaken is important even if the measurable clinical effect so far has not been as favourable as expected. Because of the relatively weak impact of oral tolerance in autoimmune diseases demonstrated so far, the treatment needed to be improved substantially.

Cholera toxin is probably the most potent immunogen known in humans and it has recently been demonstrated that oral administration of minute amounts of antigens conjugated to the non-toxic B subunit of cholera toxin (CTB), can induce tolerance among peripheral T cells as well a systemic and mucosal antibody responses (48). Furthermore, suppression of experimental autoimmune encephalomyelitis and spontaneous type I diabetes was obtained in mice by feeding MBP or insulin conjugated to CTB (49, 50) was recently demonstrated. This has now been shown also in CIA in mice. Nasal administration of small amounts of rat CII (approximately 15 μg rat CII)

conjugated to CTB induced not only protection from disease induction but also influence progression of ongoing disease. Treatment by feeeding 15 μg rat CII alone did not significantly affect the course of arthritis. Further analyses showed that anti-CII antibody production was suppressed in the CII-CTB treated mice (A. Tarkowski et. al. unpublished results and abstract at the European Workshop of Rheumatology Research, Athens 1998).

Effects of vitamin D_3 and vitamin D_3 analogues of CIA

1α,25-dihydroxycholecalciferol (1α,25(OH)2D_3), the active form of vitamin D_3, possesses immunomodulatory. Receptors for 1α,25(OH)2 D_3 are expressed on monocytes and on activated T and B cells (51, 52). Because of the strong effect exerted on calcium metabolism, systemic use of 1α,25(OH)2D_3 for treatment of immunological disorders is limited, as it may lead to the development of hypercalcemia and hypercalcuria (53-55). For this reason, new vitamin D analogues are being synthesised and investigated with regard to their potency on the immune system (56). One such analogue is MC 1288 (20-epi-1α,25-dihydroxycholecalciferol), which only differs from 1α,25(OH)2 D_3 in the altered stereochemistry at carbon 20. MC 1288 has much stronger effects than 1α,25(OH)2 D_3 on T-cell activation.

The substance inhibits the T-cell production of interleukin-2 (IL-2) (57, 58), IFN-γ (59, 60) and granulocyte-macrophage colony-stimulating factor (GM-CSF) *in vitro* and antigen- or mitogen-activated T cells incubated with 1a,25(OH)2 D_3 have decreased proliferation rates (61). The suppressive effect on IFN-γ has recently been demonstrated to be dependent on direct inhibitory effect on IL-12 induced by the inhibitory effects of 1α,25(OH)2 D_3 on the transcription factor NF-κB (62).

Because of these immunomodulatory characteristics of 1α,25(OH)2 D_3 and MC 1288 we have chosen to investigate the effects of the less hypercalcemic vitamin D analogue MC 1288 in CIA. The analogue was administered systemically at three different timepoints; (i) for 10 consecutive days before collagen (CII) immunisation, (ii) for 10 consecutive days after CII immunisation or (iii) for 7 consecutive days from disease onset. Treatment initiated either 10 days before CII immunisation or at the day of collagen immunisation effectively suppressed arthritis development. Treatment initiated at the day of arthritis onset reduced the severity of joint inflammation. Notably, doses which did not induce hypercalcemia (0,025 μg/kg MC 1288 administered intraperitoneally once daily for 10 days) significantly reduced incidence and severity of CIA (P Larsson et al, submitted).

In vivo treatment with MC 1288 diminished serum levels of antibodies to rat CII. Similarly, mitogen-induced proliferation of lymph node cells from rat CII immunised animals was suppressed.

Our experiments as well as the results from two other groups (63, 64) demonstrate that the vitamin D_3 and vitamin D_3 analogues have potential to prevent, and furthermore to suppress already established CIA. Concomitantly both T cell and B cell functions were affected. Perhaps most importantly, no side effects such as hypercalcemia and weight loss due to hypercalcemia developed at therapeutic doses.

Conclusions

The development of an ideal drug for the treatment of an autoimmune disease deals with the problem of directing the therapeutic effect to only those immune responses which are involved in the disease process. It is evident that such immune intervention is difficult to develop since the disease-inducing antigens are unknown. With such an obstacle, there is an obvious need for ideas to circumvent the problem of the elusive autoantigens. The observation that an antigen that is not in itself disease-inducing may induce protection to disease after immunisation with the disease-inducing antigen is of interest. This phenomenon is called 'bystander suppression'. Pre-immunisation of rats with methylated bovine serum albumin (mBSA) followed by intraarticular injection of sensitised rats with mBSA leads to rapid arthritis development. If sensitised rats are treated with CII they will be protected to induction of arthritis with intraarticular mBSA. (65). A similar mechanism may be operative in those RA patients who have benefited from a protective effect of oral chick CII. However, the overall clinical effect in the trials of oral CII treatment have so far not been convincing. There is a need to improve the protective effect seen in the oral CII trials. This may be brought about by the use of the strong adjuvant effects induced by CTB coupled to CII, and the suggested induction of bystander suppression to CII.

Another possiblity would be to use Th2-inducing adjuvant alum adsorbed to CII. The route of immunisation in this case would be subcutaneous or intramuscular injections (table 3).

Table 3.

Treatment of CIA	Adjuvant property	Co-administered antigen	Cytokine modulation	Effect on anti-CII antibody titers	Effects on T cell proliferation
Alum/CII (45)	Th2 stimulation	collagen II	IL4 ⇑, INFγ, IL2 and IL-10 ⇔	IgG1 ⇑, IgG2a and IgG2b ⇓	nd
Nasal cholera toxin B/CII	Both Th1 and Th2 stimulation	collagen II	IL-4, IL-6 and INFγ ⇓	IgG1 and IgG2a ⇓	nd
Vitamin D_3 and vitamin D_3 analogues(54-56)	no adjuvant used	no antigen used	nd	IgG1, IgG2a and IgG2b ⇓	decreases

nd = not done

Vitamin D_3 analogues experimentally induce a strong suppressive effect on both the development of CIA and on already developed disease. So far no experiments have been performed in our laboratory in which we have combined the immunosuppressive effect of vitamin D_3 and the bystander effect of CII. The combination of vitamin D_3 and CII may be developed for oral treatment. My hope is that such a protocol will enable us to minimise the dose of vitamin D_3 analogue needed in order to prevent the development of hypercalcemia.

Another possibility to combine specific and unspecific immunoregulation in the treatment of RA is to use the very effective inhibition of active joint inflammation seen after the treatment with anti-TNF-α monoclonals or with TNF-receptor antagonists. However, when the treatment is stopped the disease remits in many cases. One suggestion in the case of TNF-α treatment could be co-treatment with cartilage-derived antigens to induce bystander suppression as a mean to induce a more long-lasting disease protection than treatment with TNF blockade alone. For such a treatment to become effective the use of adjuvants amplifying the induction of protective Th2 (IL-4, IL-10) or Th3 (TGF-β) immunity may be needed.

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