Supplement Review

Humanized mice as a model for rheumatoid arthritis

Rüdiger Eming*, Kevin Visconti*, Frances Hall*†, Chiyoko Sekine*, Kayta Kobayashi*, Qun Chen*, Andrew Cope*‡, Satoshi Kanazawa§, Matija Peterlin§, Antonius Rijnders¶, Annemieke Boots¶, Jan Meijerink¶ and Grete Sønderstrup*

*Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California, USA
†Weatherall Institute of Molecular Medicine, Oxford, UK
‡The Kennedy Institute of Rheumatology, Imperial College, London ,UK
§Department of Microbiology and Immunology, University of California, San Francisco, California, USA
¶NV Organon, Oss, The Netherlands

Correspondence: Grete Sønderstrup, Stanford University School of Medicine, Department of Microbiology and Immunology, Sherman Fairchild Building, Room D345, 299 Campus Drive, Stanford, CA 94305-5124, USA. Tel: +1 650 723 5893; fax: +1 650 723 9180; e-mail: gretes@stanford.edu

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Chapter summary

Genetic susceptibility to rheumatoid arthritis (RA), a common autoimmune disease, is associated with certain HLA-DR4 alleles. Treatments are rarely curative and are often tied to major side effects. We describe the development of a humanized mouse model wherein new, less toxic, vaccine-like treatments for RA might be pretested. This model includes four separate transgenes: HLA-DR*0401 and human CD4 molecules, a RA-related human autoantigenic protein (HCgp-39), and a T-cell receptor (TCRαβ) transgene specific for an important HCgp-39 epitope, eliciting strong Th1 responses in the context of HLA-DR*0401.

Keywords: autoimmunity, HCgp-39, HLA-DR4 transgenic mice, rheumatoid arthritis, T-cell receptor transgenic mice

Introduction

RA is a chronic autoimmune disease affecting about 1% of the general population. RA is characterized by symmetrical inflammation of synovial joints and has often been classified with a group of organ-specific autoimmune diseases including multiple sclerosis, type 1 diabetes, and pemphigus vulgaris. RA differs from these diseases in several aspects, however, and RA patients may develop extra-arthicular disease manifestations such as rheumatoid nodules, rheumatic lung disease, and vasculitis, suggestive of a more generalized autoimmune process.

Genetic predisposition to develop RA is strongly associated with a number of human leukocyte antigen (HLA) class II alleles, which all share a collection of positively charged amino acids at positions 70–72 of the DRB1 chain, called the 'shared epitope' (reviewed by Winchester [1]). HLA class II molecules function by selecting and presenting immunogenic peptide epitopes to the $CD4^+$ T cells of the immune system. HLA class II molecules also have a major role in positive and negative selection in the thymus of the T-cell receptor (TCR) repertoire released to the periphery. It has been suggested that these

A glossary of specialist terms used in this chapter appears at the end of the text section.

mechanisms are in part responsible for the HLA-associated disease susceptibility in RA and other organ-specific autoimmune diseases [2].

There are contrasting opinions on how normal immune regulation breaks down in RA. One opinion argues that RA is a disease controlled and perpetuated by antigen-presenting cells (APC), such as dendritic cells, macrophages, and B cells, and also including conventionally nonprofessional APC such as synoviocytes and fibroblasts [3]. The other opinion stresses that $CD4^+$ T cells play an essential role in sustaining chronic autoimmunity in RA [4]. Two relatively recent murine models with spontaneous development of clinical arthritis may provide a reconciliation of these views and a better understanding of the mechanisms behind the development of RA [5,6].

The primary disease phenotype detected in several different tumor necrosis factor (TNF)-α transgenic mice with constitutive expression of human TNF has been an inflammatory arthritis similar to RA [5,7]. This phenotype is essentially preserved when these mice are backcrossed to a severe combined immune deficiency background, which lacks the development of B cells and T cells, indicating that arthritis can develop without the participation of lymphocytes. The importance of TNF-α is further supported by trials in humans, in which antagonizing TNF-α by means of anti-TNF-α monoclonal antibodies or TNF receptor antagonists is very effective in controlling arthritis symptoms, while disease activity usually rebounds if treatment is stopped [8]. It is possible that high local or systemic TNF-α production, for example in connection with infection, may function as a trigger of disease activity in RA. However, TNF-α may also exert its function indirectly through the upregulation of other cytokines [9], and it has been reported that APC populations in the rheumatoid joints have strong expression of IL-1β [10].

Spontaneous RA-like arthritis was also observed in a murine TCR transgenic model, (KRNxNOD) [6]. This TCR was originally chosen because it was specific for bovine ribonuclease in the context of $I\text{-}A^k$. The transgenic TCR$\alpha\beta$-positive cells were completely deleted in $I\text{-}A^k$ mice, however, and in attempts to rescue the transgenic $CD4^+$ T cells from deletion these mice were crossed to a number of different inbred mouse strains. Surprisingly, all the TCR transgene-positive animals developed arthritis within the first few weeks of life after the first cross to the nonobese diabetic mouse strain (NOD) [6]. These transgenic T cells were, in the context of the $I\text{-}Ag^7$ allele of the NOD, activated by a cross-reactive self-protein, glucose-6-phosphate isomerase (GPI), which is a ubiquitously expressed murine protein [11]. Transfer of purified GPI-specific autoantibodies from (KRNxNOD) mice could induce arthritis in healthy mice of the inbred balb/c and C57/B6 strains but not in mice of the inbred NOD strain, which is deficient in complement factor C′5 [12], and the (KRNxNOD) mice did not develop arthritis after backcrossing to knockout mice lacking the B-cell compartment [13]. Although GPI-specific autoantibodies seem to be essential for arthritis development in the (KRNxNOD) model, TCR transgenic CD4 T cells hold the real key to development of the disease phenotype, since the production of the GPI-specific autoantibodies is contingent on the help from CD4 T cells.

In a different TCR transgenic model system investigating the influenza hemagglutinin (HA)-specific TCR transgenic model (TS1) crossed with various transgenic lines expressing the HA as 'neo-self-antigens', Cope *et al*. have shown that the TCR $\alpha\beta$-positive T cells could change from a predominantly Th2 phenotype in TS1 single-transgenic mice to a Th1 phenotype after passing positive and negative selection in the thymus of the double-transgenic (TS1xHA) mice [14]. It is well established in several experimental models of inflammatory arthritis that the level of specific (auto)antibodies of IgG2a subtype, which is dependent on Th1 help, in the individual animal is directly correlated to the risk of developing arthritis [15–18]. The IgG subtype of the GPI autoantibodies has not been reported in the (KRNxNOD) arthritis model. It is possible, however, that the T helper cell evolution and the cytokine response pattern elicited by TCR$\alpha\beta$ transgene-positive T cells on the NOD background are the most important part of the (KRNxNOD) mice.

GPI-specific autoantibodies, which have undergone somatic mutation, have been detected in humans with RA [19]. Although it is unknown what role these autoantibodies play in disease, it is possible that components similar to those active in the (KRNxNOD) mouse may also be implicated in the autoimmune process in RA in humans.

In view of the strong genetic association between particular HLA class II alleles and RA, we assume that $CD4^+$ T cells play a significant role in the pathogenesis of this disease. $CD4^+$ T cells might supply some sort of generalized immune activation similar to the two transgenic arthritis models already mentioned [5,6]. It is possible that the activation of dendritic cells and macrophages, together with an upregulation of accessory molecules and homing receptors locally in the joints, perhaps secondary to infection, can lower the threshold for activation of autoreactive Th1 cells. From studies of autoantigen-specific $CD4^+$ T-cell responses in humans, including T-cell proliferation and different cytokine assays, it is known that not only do many patients demonstrate autoreactive T-cell responses *in vitro*, but autoreactive T-cell responses can also be detected in healthy control individuals of appropriate HLA genotype [20–22]. Why do these autoreactive T cells only rarely cause disease, and what are the differences in the immune responses of healthy individuals compared with those of patients with autoimmune diseases?

Special cytokine patterns are often taken as an indication of an activation of a unique T-cell subpopulation. We have hypothesized that the individual CD4+ T-cell epitopes of a given autoantigen in the setting of one particular HLA class II allele in a healthy *in vivo* study subject would activate a distinct CD4+ T-cell population. To test this hypothesis, we have studied cytokine responses in healthy HLA-DR4 transgenic mice after immunization with intact protein antigen, followed by cytokine measurement after *in vitro* re-stimulation with the individual peptide epitopes. Although immunization with antigen in incomplete Freund's adjuvant (IFA) might bias the cytokine responses to a certain degree, comparative studies using other forms of immunization with antigen-pulsed dendritic cells or DNA vaccination should ultimately resolve this problem.

We chose to analyze the CD4 T-cell immune responses to the human cartilage autoantigen, HCgp-39, in the setting of the RA-associated HLA-DR*0401 (DRA*0101/DRB1*0401) HLA class II molecule [23]. To augment the autoreactive T-cell responses of these mice, a TCRαβ transgene was added to the model. The TCR construct was produced from a selected T-cell hybridoma specific for a HCgp-39 peptide epitope, which induced a significant IFN-γ response.

Development of HLA class II transgenic mice

The original HLA class II transgenic mouse model was designed to serve as an *in vivo* animal model in which several aspects of the human CD4+ T-cell immune responses could be studied after immunization with either endogenous or exogenous protein antigen [24]. Transgenic mice with the RA-susceptible DR*0401 allele and the RA nonassociated DRA/DRB1*0402 (DR*0402) allele, which had IDE in place of the QKR sequence of the 'shared epitope', were produced using cDNA matching the entire human coding sequences for the DRA and DRB1 chains expressed under the direction of the I–E alpha promoter [25].

To obtain sufficient selection of CD4+ T cells, it was necessary to introduce a correctly expressed human CD4 transgene and to delete the murine major histocompatibility complex class II genes [24]. This was achieved by cross-breeding with a human-CD4 transgenic line provided by Dr D Littman, and the murine class II-negative Aβ line from Dr D Mathis and Dr C Benoist [26,27]. After these changes, the cell surface expression of the HLA-DR*0401 molecule increased two to three times, and the CD4+ T-cell counts increased accordingly [24]. However, all our HLA class II transgenic mice, which were carefully selected from between six and 15 different transgenic founder lines per HLA specificity, had preserved normal lymphoid architecture and correct tissue-specific expression of the introduced HLA molecules in the thymus, the lymph nodes and the spleen. These mice had normal CD4+ T-cell function and, similar to HLA-DR4+ humans, they did not develop spontaneous autoimmune diseases [24].

Choice of human autoantigen

The human cartilage protein HCgp-39 was chosen as a model autoantigen because it was a proven target for T-cell autoimmunity in RA patients [23,28]. A significant fraction of RA patients had shown signs of previous T-cell activation specific for a number of the immunogenic peptides from the protein, suggesting that it might be a possible target for future immunotherapy in RA. Our choice of prototype antigen, however, did not imply that HCgp-39 was supposed to have a unique role in the pathogenesis of RA.

Epitope mapping of HCgp-39 protein in HLA-DR*0401 and HLA-DR*0402 transgenic mice

CD^{4+} T-cell epitope mapping after immunization of DR*0401 and DR*0402 transgenic mice with recombinant HCgp-39 in IFA was performed using the T-cell hybridoma technique, which is described in detail in Cope *et al*. [23]. These experiments, involving 16 HLA-DR*0401 and 12 HLA-DR*0402 transgenic mice of appropriate genotypes, revealed three major immunogenic HCgp-39 epitopes (peptides 100–115, 262–277, and 322–337) in DR*0401 mice and two major epitopes (peptides 22–37, and 298–313) in DR*0402 mice [23]. A number of minor HCgp-39-specific T-cell epitopes were also identified [23]. Investigation of peptide–HLA complex stability for the major immunogenic HCgp-39 epitopes showed that the immunogenicity of DR*0401 and DR*0402 peptide epitopes was strongly related to the kinetic stability in all conditions, from the acidic endosomal compartment in the presence of the peptide editor HLA-DM to the neutral cell surface conditions [29].

HCgp-39-specific cytokine production in HLA-DR*0401 transgenic mice

Early cytokine studies in DR*0401 and DR*0402 transgenic mice had shown that intact HCgp-39 protein elicited strong IFN-γ and TNF-α responses in DR*0401 transgenic mice, while fairly small IFN-γ responses were detected in the DR*0402 transgenic mice [23]. TNF-α responses were not detected in the DR*0402 transgenic mice after *in vivo* immunization with HCgp-39 [23]. The intact HCgp-39 protein, as well as the individual HCgp-39 peptides, induced only small amounts of IL-2 in DR*0401 transgenic mice (Hall F, manuscript in preparation).

Two immunodominant peptide epitopes (peptides 100–115 and 322–337) appeared to be responsible for the majority of the IFN-γ elicited in response to HCgp-39 immunization. The Th2-type cytokine IL-5, however, was predominantly generated by peptide 100–115, while moderate amounts of TNF-α and intermediate levels of IL-10 could be driven by either peptide 100–115 or peptide 322–337 (Hall F, manuscript in preparation). The third

major epitope (peptide 262–277) elicited small amounts of each of the five cytokines tested, and the significance of this reactivity pattern is unknown.

The bulk of the TNF-α was produced in response to the two minor peptide epitopes, peptides 256–271 and 334–349 (Hall F, manuscript in preparation). Considering the low frequencies of T cells responding to these two peptide epitopes [23], this response was (on a per-cell basis) approximately 50 times higher than the TNF-α produced in response to peptides 100–115 and 322–337. On a per-cell basis, peptides 256–271 and 334–349 elicited about the same IFN-γ levels as peptide 322–337, but no IL-5 or IL-10 responses were detected after re-stimulation with either of these 'minor' peptides (Hall F, manuscript in preparation).

The two T-cell epitopes 256–271 and 334–349 had been selected for further studies because they had elicited T-cell proliferative responses in the majority of both the HLA-DR4-positive RA patients and the HLA-DR4-positive human control subjects studied [23] (Sønderstrup G, unpublished results, 1998). Peptides 256–271 and 334–349 may therefore activate a unique subset of CD4 T cells. However, it should be emphasized that HCgp-39 functioned as a foreign antigen in the HLA-DR*0401 transgenic mice, while it was a self-protein in humans.

Cytokine responses after immunization with synthetic peptide versus intact protein antigen

Since a major objective was to identify a TCR for production of TCR transgenic mice in which disease might be induced, we chose to focus on peptide epitope 322–337 that exhibited a Th1 cytokine pattern. Figure 1a shows the IFN-γ response of four individual HLA-DR*0401$^+$/human CD4$^+$/murine I-Aβ$^{-/-}$ mice after immunization with recombinant HCgp-39 protein in IFA followed by *in vitro* re-stimulation with medium only (control), intact HCgp-39 protein (20 μg/ml), and the specific peptide 322–337 (10 μg/ml) for 24, 48, and 74 hours. Three of the four mice produced high IFN-γ levels to both protein and peptide, while mouse 1 responded much less (Fig. 1a).

In a similar study of four additional DR*0401 transgenic mice that were immunized with the synthetic peptide 322–337, 100 μg in IFA (Fig. 1b), lymph-node T cells preferentially responded with high levels of IFN-γ to the synthetic peptide itself. The IFN-γ responses to intact HCgp-39 protein were about 10 times lower (Fig. 1b).

These data suggest that the responding CD4+ T-cell population activated *in vivo* following immunization with a synthetic peptide may be different from the T-cell population responding to the same amino acid sequence if processed from the native protein antigen. T-cell clones that recognize the same peptide–HLA complex, which will only respond after *in vitro* re-stimulation with the synthetic peptide but not the intact protein antigen, have been described earlier as type b T cells [23,30]. In a recent study of antigen processing and presentation of the immunodominant HCgp-39 epitopes using HLA-DM-positive and HLA-DM-negative human APC, we have also shown that intact HCgp-39 protein must be processed via the endosomal compartment in the presence of HLA-DM molecules to induce activation of the peptide 322–337-specific T-cell hybridoma clones [31].

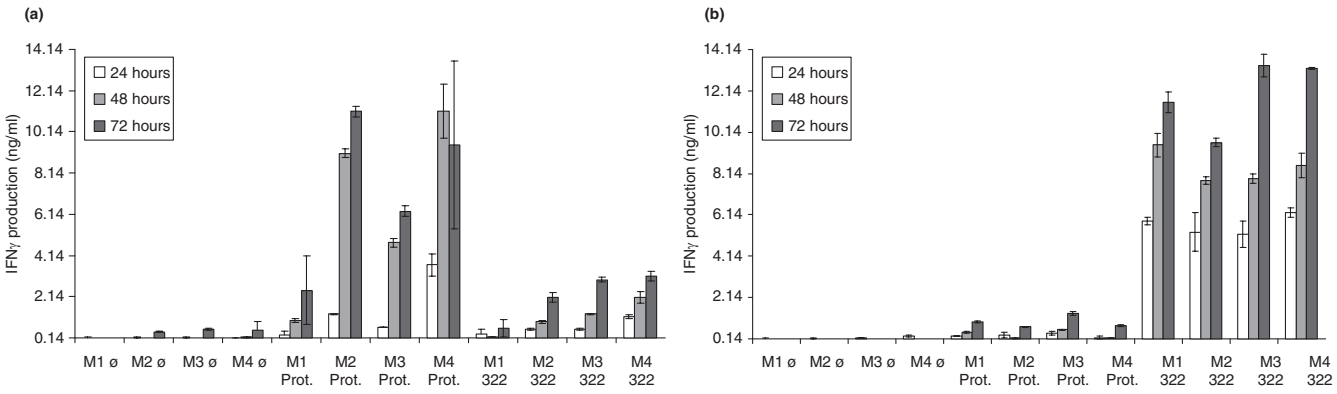
TCR transgenic mice specific for peptide 322–337 in the context of HLA-DR*0401

Detailed studies of more than 10 T-cell hybridomas, including antigen titration, N-terminal and C-terminal truncation studies as well as a complete set of alanine-substituted peptides across the wild-type sequence of peptide 322–337, revealed that although all the peptide 322–337-specific T-cell hybridomas recognized the same peptide–HLA complex, they fell into two groups with distinct response patterns (Hall F, manuscript in preparation). Approximately one-half of the T-cell hybridomas were completely unaffected by alanine substitutions outside of the 9mer core epitope. The second group of the peptide 322–337-specific T-cell hybridomas produced less IL-2, and these hybridomas usually required a relatively high antigen concentration. The latter group of T cells was sensitive to both alanine substitutions in the 9mer core region of the peptide that bound in the groove of the HLA-DR*0401 molecule and to substitutions in the N-terminal overhanging amino acids. These residues were presumably TCR contact residues since they only influenced T-cell responsiveness and had no influence on peptide–HLA binding (Hall F, manuscript in preparation).

We consequently chose to produce two separate TCR αβ constructs using a T-cell hybridoma representing each of these two different response patterns. The two TCR α-chain and TCR β-chain constructs were produced using the pTαcass and the pTβcass kindly provided by D Mathis and C Benoist [32]. The first TCR αβ construct, TCR/18B1, which was produced using a T-cell hybridoma from the N-terminal overhang-dependent group, was TCRVα11, Vβ14. Six TCR transgenic founders transmitting at least the TCR β-chain transgene were obtained. The 18B1/TCR β-chain was selected both by I-A^q and DR*0401$^+$ mice. Three of these lines have been tested on the DR*0401 background and they all responded to both the nominal peptide and the HCgp-39 protein.

FACS analyses demonstrated that the transgenic TCRVβ-chain was expressed on more than 95% of CD4$^+$ T cells. Less than 1% of the T cells expressed other TCRV β-chains. However, the transgenic T cells did not show an *in vivo* activated phenotype. The founder lines of TCR/18B1, which are still in an early stage of evaluation, will be tested

Figure 1



(a) IFN-γ responses following immunization with HCgp-39 protein. The IFN-γ responses of four different DR*0401 transgenic mice immunized with recombinant HCgp-39 in incomplete Freund's adjuvant following *in vitro* re-stimulation with either intact protein or immunodominant peptide epitope 322–337. **(b)** IFN-γ responses following immunization with peptide 322–337. The contrasting IFN-γ responses elicited by either intact protein antigen or peptide 322–337 after immunization of four similar DR*0401 mice with the synthetic peptide 322–377 itself.

for incorporation of the appropriate TCRVα-chain by means of polymerase chain reaction using specific primers. DR*0401/peptide 322–337 tetramers are under development and will be used to determine the exact levels of expression of the transgenic TCRαβ combination.

The second TCR transgene, 14H2, was TCRVβ6. Unexpectedly, the 14H2/TCR was only selected on an I-A^q-positive background. Several transgenic founder lines expressing and transmitting the TCR transgene were obtained. When these mice were backcrossed to the DR*0401/human CD4/I-$Aβ^{-/-}$ genetic background, however, no Vβ6-positive T cells (0–1%) could be detected in the periphery. Non-TCR transgenic littermates with the full DR*0401+/human CD4+/I-$Aβ^{-/-}$ genotype usually express between 2 and 5% Vβ6-positive T cells. It is currently unclear whether the absence of Vβ6-positive T cells in these mice is due to a lack of positive selection or due to a complete deletion of the T cells expressing the transgenic TCRVβ6 chain by thymic negative selection.

To rescue the 14H2/TCR transgene, founder lines that express transgenic TCRVα and Vβ chains on the I-A^q genetic background will be backcrossed to an HLA-DR*0401+ transgenic line on the NOD genetic background, which is less efficient in negative selection. Inhibition studies have shown that the 14H2 T-cell hybridoma exclusively interacts with the human CD4 molecule (Sønderstrup G, unpublished results, 2001), and separate attempts to rescue thymic selection of the transgenic 14H2/TCR are in progress by crossing the 14H2/TCR transgene onto a DR*0401+/I-$Aβ^{-/-}$/DBA/1J background without human CD4. Finally, the 14H2/TCR transgene will also be crossed with HLA-DR*0405 transgenic mice on the NOD background. The HLA-DR*0405 allele, which is closely related to the DR*0401 allele, is also associated with susceptibility to RA [2].

HCgp-39 transgenic mice

The human HCgp-39 protein [33] and the murine counterpart, Brp39 [34], show more than 80% sequence homology, but they differ significantly in the areas that are found to be important for T-cell recognition in HLA-DR*0401 transgenic mice [24]. We therefore produced transgenic mice carrying the human HCgp-39 protein expressed under the murine collagen type II promoter (ColI), which directs expression to the synovial joints and the eye [35].

An optimal founder line for the ColI-HCgp-39 mice was identified after intercrossing with DR*0401+/human CD4+/I-$Aβ^{-/-}$ mice by following the HCgp-39 protein content of synovial joint cartilage over three generations. Since the ColI-HCgp-39 transgene functioned as a neo-self-antigen, the DR*0401 transgenic mice were tolerant to HCgp-39. However, they did break tolerance and developed specific antibodies of IgG1, IgG2a, and IgE isotypes after sequential immunization with HCgp-39 in complete Freund's adjuvant followed by immunizations in IFA (Hall F, manuscript in preparation). We expect to find a qualitative difference in the HCgp-39-specific immune responses of DR*0401/ColI-HCgp-39 double-transgenic mice compared with DR*0401 single transgenic mice.

Future prospects: strategies for triggering *in vivo* activation of the TCR αβ? transgenic T cells

FACS analysis using anti-CD25, anti-CD44, and anti-CD69 monoclonal antibodies does not indicate increased levels of activated CD4+ T cells in the DR*0401+, 18B1/TCR double-transgenic mice. One major objective is thus to activate the CD4+ T cells carrying the transgenic

Table 1

Genotypes of experimental animal groups

Group	Genotype	
1A	DR*0401$^+$/human CD4$^+$/Aβ$^{-/-}$, 18B1/TCR, CoII-HCgp-39, TET-CoII-TNF-α	
1B	DR*0401$^+$/human CD4$^+$/Aβ$^{-/-}$, 18B1/TCR, CoII-HCgp-39	Control group
2A	DR*0401$^+$/human CD4$^+$/Aβ$^{-/-}$, 18B1/TCR, CoII-HCgp-39	HCgp-39 in IFA
2B	DR*0401$^+$/human CD4$^+$/Aβ$^{-/-}$, 18B1/TCR, CoII-HCgp-39	Control group
3A	DR*0401$^+$/human CD4$^+$/Aβ$^{-/-}$, 18B1/TCR, CoII-HCgp-39, Ii-HCgp-39	
3B	DR*0401$^+$/human CD4$^+$/Aβ$^{-/-}$, 18B1/TCR, Ii-HCgp-39	
3C	DR*0401$^+$/human CD4$^+$/Aβ$^{-/-}$, 18B1/TCR	Control group

The table shows the detailed genotypes of the different groups of multitransgenic mice that will be used to explore the influence of joint-specific CD4 T cells in the development of inflammatory arthritis in the humanized *in vivo* mouse model. CoII, collagen type II promoter; HCgp39, human cartilage glycoprotein 39; IFA, incomplete Freund's adjuvant; Ii, invariant chain; TET, tetracycline-inducible TNF-α transgene.

TCRαβ combination of these mice *in vivo*, and this might be achieved by simple immunization with the HCgp-39 protein or the cognate peptide. However, an immunization approach may be hampered by processing problems or by reverse effects of other HCgp-39 T-cell epitopes. Two separate strategies will therefore be pursued in parallel. The first uses transgenic mice carrying a tetracycline-inducible TNF-α transgene expressed under the murine CoII, which theoretically should induce local activation of the APC similar to the TNF-α transgenic mice [5]. Founder mice are currently under selection. A second transgenic approach will target the peptide 322–337 sequence to the endosomal compartment of professional APC. This approach will use a DNA construct that substitutes the oligonucleotide sequence of peptide 322–337 for the CLIP sequence of the murine invariant chain and insert this cDNA into a modification of a murine invariant chain cassette vector developed by D Mathis and C Benoist [36].

Different combinations of these humanized mice will be used to explore whether clinical arthritis can be induced in mice by triggering the APC with local TNF-α release (group 1A), by activating the transgenic TCR with its specific antigen/peptide as a vaccination in CFA (group 2A), or by *in vivo* activation of the TCR transgene by crossing with a transgenic line (group 3B), which expresses the cognate peptide as a transgene under the invariant chain promoter. Mice of the genotypes show in Table 1 will be monitored in groups of 10 mice for development of clinical arthritis up to 30 weeks of age.

Assuming that the control mice (groups 1B, 2B, and 3C) will not develop disease spontaneously, occurrence of arthritis in mice of group 1A will suggest a major role for local APC in the development of arthritis. Arthritis after immunization of group 2A mice will imply a major role for CD4 T cells in arthritis development. Arthritis in group 3A mice, which carry the HCgp-39 epitope as a neo-self-antigen both in the APC (expressed under the invariant chain promoter) and locally in the joints (expressed under the CoII promoter), but not in group 3B mice, which only express the HCgp-39 epitope in the APC, will indicate that joint antigen-specific CD4 T cells may be very important in disease induction. Arthritis in group 3B mice, but not in group 3A mice, will suggest that high local expression of autoantigen plays a key role in keeping self-reactivity in check. Finally, arthritis occurrence in both group 3A and group 3B mice will indicate that generalized immune activation plays a major role in RA.

Concluding remarks

This HLA-DR*0401 transgenic mouse model was designed to provide an *in vivo* animal model that would faithfully replicate certain aspects of an HLA-DR*0401-positive human CD4 T-cell immune system, and therefore these mice were not expected to develop arthritis or other autoimmune disease manifestations spontaneously. The addition of a neo-self-antigen, the human autoantigenic protein HCgp-39, which in humans can be produced by several different cell types in many different tissues during inflammatory conditions, was expected to provide antigen specificity to the autoimmune responses of these humanized mice. As outlined in Table 1, the further addition of the HCgp-39-specific TCR transgene was anticipated to enlarge the autoantigen-specific Th1 response to this human neo-self-antigen. A small population of TCR transgene-positive cells, which may have escaped negative selection in the thymus but later encounter their specific peptide presented in the periphery in the context of HLA-DR*0401 molecules, may then be activated and expanded

in the lymph nodes and the spleen. These humanized mice would be expected to reproduce some of the most important features of human RA and provide a model of inducible arthritis, which alone or in connection with cell transfer experiments can be used to develop and pretest vaccine-like immunomodulatory therapies for AR in humans.

Glossary of terms

Coll = collagen type II promoter; DM = HLA-DM, a non-peptide-binding HLA class II molecule, which facilitates exchange of peptides of already formed peptide/HLA class II complexes, mostly in the acidic endosomal compartment of the antigen-presenting cell; GPI = glucose-6-phosphate isomerase; HA = influenza hemagglutinin; HCgp-39 = human cartilage glycoprotein 39; HLA-DR*0401 = DRA, DRB1*0401; HLA-DR*0402 = DRA, DRB1*0402; HLA-DR*0405 = DRA, DRB1*0405; IFA = incomplete Freund's adjuvant; NOD = nonobese diabetic (mouse); TS1 = influenza hemagglutinin-specific TCR transgenic model.

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