Preface

There is an ever growing number of autoimmune diseases ranging from systemic and multi-organ specific to organ-specific disorders. Worldwide, millions of people are suffering from autoimmune diseases. Many of those diseases dramatically change the life of patients and may even become life threatening. Morbidity and mortality result from the involvement of essential organs. The usual live-long treatment of autoimmune patients are very expensive and the illness is often debilitating. Unfortunately, some patients have to suffer for years until a correct diagnosis leads to appropriate or effective therapy. At least in some cases, an earlier diagnosis would improve the outcome of therapy.

What has been done, what has to be done, what can be done, now and in the future, in order to improve the diagnosis and therapy of autoimmune patients? For this purpose it is necessary to investigate the etiology and pathogenesis of the different forms of autoimmune diseases. The factors and the complex mechanisms that are involved in the development of pathological autoimmunity are incompletely understood. However, it appears to be likely that genetic as well as environmental factors are responsible for the induction, development and progression of most autoimmune diseases. Although some of the pathological aspects can be explored by analysis of human body fluids and tissues and by epidemiological studies the puzzle of the etiopathogenesis of autoimmune diseases cannot readily be analyzed without appropriate animal models. It is not astonishing therefore, that the fourth AAA volume has focused on animal autoimmune models for exploring the pathogenesis and therapy of autoimmunity in mice, the role of natural and pathogenic autoantibodies, the molecular mechanisms of xenobiotic-induced autoimmunity as well as the impact of various genes on disease development.

Another focus is on the relevance of autoantibodies in human disease as well as the genetic factors in SLE and rheumatoid arthritis (RA). The identification of PADI4, a gene encoding peptidylarginine deiminase type 4, as a RA susceptibility gene may establish ties between breaking tolerance by post-translational protein-modifications and the induction of autoantibodies to the pathogenesis and potential molecular targets for therapeutic intervention. Many open questions remain to be solved by studies on men as well as animal models.

The editors

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Pathogenesis and Therapy of Autoimmunity in Experimental Mouse Models

Autoimmunity in men and mice: An introduction

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The revolutionary techniques of modern molecular and cellular biology enhance almost daily our knowledge about immunity and autoimmunity in men and experimental animals. E.g. using linkage studies, gene arrays, multiple parameter assays combined with SNPs, PCR and cloning techniques we are on the way to gain an exceptional view on the genetic background of autoimmune patients, the development of immune cells, the genes involved in immune and autoimmune responses, the origin of the diversity of antigen receptors, the cross talk between the players of the immune system mediated by cytokines, as well as the mechanisms leading to activation or downregulation of immune reactions. Our fragmentary puzzle of the immune system is going to form a fascinating picture of a master piece of evolution. Although many of these aspects were achieved by analysis of human tissues, the etiopathogenesis of autoimmune diseases cannot readily be analyzed without appropriate animal models.

Since the late 1950s it is known that some inbred mice develop spontaneously a disease similar to human systemic Lupus erythematosus (SLE) (1-3). E.g. the (NZB x NZW)F1 hybrids underwent changes remarkably similar to those of human lupus nephritis (1). Other SLE strains followed such as the MRL and the BXSB mice (2,3). All these strains have in common that they originate from complex cross-breeds.

The MRL mouse originated from a series of crosses involving inbred strains AKR/J, C57BL/6, C3H/Di, and LG/J. In the 12th generation, a portion of the MRL mice (MRL/l) expressed lymphadenopathy. The other portion did not (MRL/n). Reciprocal back-crosses of MRL/n (which were renamed at F28 as MRL/MP-+/+) with MRL/p indicated that a single recessive gene was responsible for lymphoproliferation (*lpr* gene). The *lpr* gene was transferred by a series of cross-intercross breadings to MRL/MP-+/+ mice resulting in the MRL-*lpr/lpr* mice. In addition, the *lpr* gene was transferred to other standard inbred mice including for example AKR, Balb/c, C57BL/6 and SJL. Later the *lpr* gene was identified as the gene encoding the FAS receptor, a receptor

involved in programmed cell death, and mutations were found in the *lpr* gene inhibiting the FAS/FASL signal transduction cascade (e.g. 4).

The NZB strain was originally bred for coat color already from an unknown background. Based on (NZB x NZW)F1 hybrids the NZM2310 mouse strain was established.Three genomic intervals containing the SLE-susceptibility genes (Sle1, Sle2, Sle3) were identified via a genome-wide analysis of SLE susceptibility in a (NZM2410 x C57BL/6)F1 x NZM2410 backcross, and transferred independently on a C57BL/6 background to produce three congenic strains (B6.NZMc1 carrying Sle1, B6.NZMc4 carrying Sle2, and B6.NZMc7 carrying Sle3). By combining the Sle1, Sle2, and Sle3 loci into a triple congenic strain, it was shown that these loci contain the minimal set of genes sufficient to reconstitute a fully penetrant SLE pathogenesis in these mice (5). Remarkably, one region of the genome, telomeric chromosome (chr) 1 in the mouse and its syntenic equivalent 1q21-44 in humans, has shown strong linkage in all human studies and in all genome scans conducted in the (NZB x NZW)F1 model and its derivative, the NZM2410 strain. In addition to linkage studies, association/case-control or gene disruption studies have shown that genes located in this region, such as those encoding for FcγRIIA, and FcRγIIIA, ADPRP, and SAP, play a role in SLE susceptibility (6-11).

It is far beyond any capability to even summarize our present knowledge about all these spontaneous autoimmune animal models in this section related to autoimmune animal models. Some human susceptibility genes identified by linkage studies e.g. at 1q23 will be discussed later in the genetic factors and autoimmunity session. Here, we focus on more recent, in part controversially discussed autoimmune animal models, including for example the p21- and the Ro60 knock out mouse, or a break of tolerance via immunization with apoptotic material.

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Novel autoimmune models: Lessons from recent transgenic and knock in animals

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Patients suffering from autoimmune diseases such as scleroderma, polymyositis, and systemic lupus erythematosus (SLE) frequently produce autoantibodies against highly conserved ribonucleoprotein particles. Interestingly, specific antibodies are associated with distinct rheumatic diseases e.g. antibodies to nucleolar components are often found in patients with Scleroderma while antibodies to components in tRNA-synthetase complexes are commonly found in patients with polymyositis. The occurrence of autoantibodies is also a unifying feature in patients with SLE [1]. Up to 30 % of these patients produces IgG autoantibodies to the autoantigens Ro and La [2]. A higher prevalence of anti-La and anti-Ro autoantibodies occurs in individuals suffering from primary Sjögren’s syndrome (pSS). Nearly 90 % of patients with pSS produces autoantibodies with specificity for La and Ro. Perhaps, the highest prevalence of these autoantibodies is in mothers who have given birth to children with neonatal lupus (NL) [3]. About one third of patients having both serum specificities also produce antibodies to dsDNA [4]. Deposition of dsDNA containing immune complexes in kidneys can cause a glomerulonephritis [4]. The occurrence of anti-Ro antibodies is highly associated with photosensitivity and photosensitive skin lesions, particularly those of subacute cutaneous LE [5,6].

The La- and Ro60 antigen system

Both, the La and the Ro antigen are RNA binding proteins. The major portion of both proteins is present in the nucleus. However, a minor portion can be found in the cytoplasm. In the cytoplasm but not in the nucleus the Ro antigen interacts with one of several Ro RNAs known as YRNAs [7-10]. Although the number and sequence of the respective YRNAs depends on the species the Ro protein/Y RNA complex is conserved evolutionarily. Orthologues have

been described in mammals, *Xenopus laevis*, and *Caenorhabditis elegans*. Curiously, a Ro RNP was even found in the radiation-resistant eubacterium *Deinococcus radiodurans* [11-15]. In this bacterium, Ro protein contributes to the resistance to environmental stress including UV irradiation. Although the function of Ro RNPs still remains unclear, the binding of the Ro protein to misfolded 5S RNAs led to the hypothesis that Ro60 may be involved in RNA quality control [9,16].

The La antigen is also associated with a series of small RNAs. It binds to almost all precursor RNAs transcribed from RNA polymerase III including for example the precursors of 5S, tRNAs and U6 snRNA. This interaction occurs with the 3'-oligo(U)-sequence that is common to all RNA polymerase III precursors [17]. However, La protein can also bind to some RNA polymerase II transcripts including some cellular mRNAs [18]. Besides cellular RNAs, La protein can also bind to some viral RNAs e.g. to the VA RNAs encoded by adenovirus or the EBER RNAs encoded by the Eppstein Barr virus [19,20]. The function of these complexes is unknown. There is growing evidence that La protein has different nuclear and cytoplasmic functions. Aside a nuclear function in transcription/processing of RNA polymerase III, La protein seems to be involved in translation of a special class of mRNAs. The latter function was first observed for poliovirus mRNA [21]. It is well known that cellular eucaryotic mRNAs are capped with a m7-G cap at the 5'-start. The capping occurs in the nucleus. Poliovirus like many RNA viruses replicates in the cytoplasm. Therefore, such viral mRNAs are not capped with the classical m7-G cap. However, this cap structure is a prequisite for translation according to the 5'-scanning mechanism [22]. Furthermore, many viral mRNAs contain additional putative initiation AUG codons upstream of the actual initiation site. These upstream ORFs do also interfer with a 5'-scanning mechanism. For these reasons such mRNAs contain an internal ribosomal entry site (IRES) which allows the ribosome to identify the correct initiation site. Although the final mechanism of internal initiation of translation still remains unclear, a series of cellular proteins seem to be involved in this process. Interestingly, all of these factors are nuclear proteins including for example some hnRNP binding proteins, the polypyrimidine tract binding protein (PTB) and the La protein [e.g. 21,23]. This function of La protein was first described for poliovirus but there is now a growing list of viruses that interact with La protein including for example hepatitis C, HIV, rhinoviruses and many more [e.g. 24-26]. In addition to viral mRNAs, La protein was found to bind to IRES elements in cellular mRNAs including for example in the mRNA encoding the human immunoglobulin heavy chain-binding protein (BiP/GRP78), the double minute gene mdm2 and the X-linked inhibitor of apoptosis (XIAP) [27-29]. A functional interaction of La protein was also reported with the class of 5'-TOP mRNAs which mostly encode for proteins required for protein synthesis prior to cell division [30]. During translation La and Ro protein may work together in a complex [31].

Until recently, the autoantigens Ro and La were only known as targets of an autoimmune response in patients with systemic autoimmune diseases. There was no evidence for a direct link of these autoantigens to a role in the

development of systemic autoimmunity. Recently Xue et al. [32] reported that Ro60 knock out mice develop a lupus like syndrome. Moreover, we established mice transgenic for a mutant form of the La antigen which we isolated originally from a cDNA library made from peripheral blood mononuclear cells (PBMCs) of a patient with SLE and Sjögren's syndrome [33]. Unexpectedly, these mice also developed a lupus like disease leading us to the hypothesis that an impaired expression of one of these nuclear antigens could perhaps predispose to the development of systemic autoimmunity.

Regulation of the cell cycle

In general, an immune response to a foreign antigen involves a sequence of T and B cell activation, clonal expansion, differentiation into effector cells and, finally, regulated cell death [34,35]. A small fraction of cells that escapes apoptosis constitutes self-renewing, long-living memory cells. Although not finally proven, similar principles seem to work in case of an autoimmune response [36]. However, differences in the kinetic and homoestasis of an autoimmune response must and do exist in an autoimmune patient as well as in autoimmune prone animals.

A common feature of lupus prone mice is a marked accumulation of activated memory B and T cells [37,38] which are resistant to proliferation and apoptosis. Notably, these cells are arrested in G0/G1-phase of the cell cycle [38]. Interestingly, TCR-, mitogen-, and superantigen-mediated apoptosis of mature T cells requires a progression through several cellular divisions [39-41]. A blockade of the cell cycle using antisense oligonuceotides to cyclin B results in an inhibition of TCR signal-mediated apoptosis [42]. Consequently, blocking the progression of the cell cycle at G0-G1 phase could be a major reason for the apoptosis resistance and accumulation of the activated memory cells in systemic autoimmunity.

A series of proteins are involved in the regulation of the cell cycle including positive and negative regulators. Among them are the cyclins, cyclin-dependent kinases (CDKs) and the cyclin-dependent kinase inihibitors (CDKIs). Notably, certain CDKIs including p21(WAF-1/CIP-1) are upregulated in the apoptosis resistant activated memory cells found in autoimmune prone mice [38].

Autoimmunity in animal models related to the cell cycle inhibitor p21, the Ro60 or the La antigen

The aim of this article is not to give an overview on all the known, more or less well understood, autoimmune prone mouse models or the increasing numbers of genetically manipulated and more or less degenerated mice. Here, we are going to focus on the three recently described autoimmune mice models

including a p21 knock out mouse [43], the Ro60 knock out mouse [32,44], and the mouse transgenic for a mutant form of the autoantigen La/SS-B. At a first glance, these three animal models seem to have little in common. However, we try to provide some first inside for a potential common mechanism in these autoimmune models and their potential link to systemic autoimmunity in SLE patients.

Inhibition of spontaneous systemic autoimmunity by p21 knock out

CDKIs are negative regulators of cyclin-CDK complexes. They have been grouped in the two families Ink4 and CIP/KIP [45,46]. The ink4 members (p16/Ink4A, p15/Ink4B, p18/Ink4C, and p19/Ink4D) form complexes with CDK4 and CDK6 and, thus, block the cell cycle at G1 to S-phase. The pancyclin CIP/KIP proteins (p21/CIP-1/WAF-1, p27/KIP-1, and p57/KIP2) bind the cyclin/CDK complexes at all stages of the cell cycle [45-49]. A major player in both regulation of the cell cycle and apoptosis by promoting cell cycle arrest, inhibiting proliferating cell nuclear antigen (PCNA), p53 and procaspase 3 is the CDKI p21.

In order to proof the hypothesis that high levels of expression of p21, and the occurrence of long living memory cells as the result of the cell cycle arrest, is responsible for the development of systemic autoimmunity in autoimmune prone mice, Lawson et al. [43] decided to cross p21 knock out mice with the autoimmune prone mice BXSB. According to their hypothesis, downregulation of p21 should protect the autoimmune prone mice against spontaneous development of autoimmunity. Indeed, their experimental data shows that deletion of p21 significantly reduces serologic, cellular, and histological disease manifestations and increases the survival of lupus prone BXSB mice. In parallel, they observed a reduced accummulation of proliferation- and apoptosis-resistant T and B cells.

Taken together, this data suggests that upregulation of p21 and the occurrence of proliferation- and apoptosis resistant T and B cells contribute significantly to the autoimmune and inflammatory processes that are critical for disease pathogenesis, at least in this spontaneous autoimmune mouse model.

It should be mentioned that the result of Lawson et al. is in contrast with an initial report by Balomenos et al. [50]. In this paper it was shown that female, but not male, p21 -/- mice of mixed 129/Sv X C57BL/6 (129 X B6) background develop a severe lupus like disease. However, subsequent analyses with a different group of p21 -/- mixed 129 X B6 mice could not confirm this original data [51,52]. Meanwhile, several papers have documented that mice with a 129 X B6 mixed genome develop signs of systemic autoimmunity with low levels of glomerulonephritis (GN), especially female animals of advanced age [52-56]. This should be a “caveat” including for the Ro60 knock out mice described next, and also for other 129 X B6 knock out mice. Signs of systemic autoimmunity in such mice must be

carefully controlled by a sufficient number of back crosses to attain genetic homogeneity.

A lupus like syndrome in mice lacking the Ro60 autoantigen

In lupus patients, photosensitivity occurs in up to 90% of patients with anti-Ro antibodies [6]. Moreover, maternal anti-Ro antibodies that cross the placenta are highly associated with photosensitive skin lesions in infants which disappear in parallel to the disappearance of the anti-Ro antibodies [57,58]. Interestingly, the prokaryotic orthologue of the Ro antigen in *D. radiodurans* is important for the survival of the eubacterium after UV irradiation. In line with this putative role of Ro60 in UV sensitivity, the absence of Ro60 in the recently described Ro60 -/- mouse results in a significant photosensitivity [32]. However, both wild-type and Ro60 -/- mice were less sensitive than the backcrossed mice. Unexpectedly, the mice lacking the Ro protein showed an increased mortality. Histologic examination revealed a membranoproliferative glomerulonephritis (GN) in these mice. However, the GN and the increased mortality were only found for the F2 and F3 hybrids of the 129 X C57BL/6 F1 hybrid mice, while after three backrosses to C57BL/6 mice, the Ro60 -/- mice no longer exhibited an increased mortality compared with their wild-type counterparts. Nonetheless, compared to wildtype mice the autoimmune response in the Ro60 -/- appeares to be more severe than in other 129 X B6 crosses and, thus, could be specific. The mechanism, how the absence of Ro60 can trigger an autoimmune response, remains unclear. It was hypothesized that the release of intracellular components of Ro60 -/- cells that occurs normally during cell turnover triggers the autoimmune response. According to this model, the presence of misfolded 5S rRNAs could cause a break of tolerance by exposing normally cryptic determinants to the immune system.

A lupus like syndrome in mice transgenic for a mutant form of the La autoantigen

Detection of a mutation in the La gene of an anti-La patient

Native human La protein has a molecular weight of approximately 50 kD. It consists of two domains (Fig. 1A). The N-domain (29 kD) binds to small RNAs. The C-domain (25 kD) contains a nuclear location signal (NLS) and a dimerization site (DIM). A protease sensitive peptide which is encoded by exon 7 of the La gene connects the two domains. Within exon 7 (Fig. 1B, nts 1051-1059), the La sequence contains an oligo$(A)_8$-region which encodes the aa 190-192 (KKN).

In previous studies, we isolated a La cDNA with a deletion of an (A)-residue in the oligo$(A)_8$-region (double headed arrow in Fig. 1B; c,d). The

frame shift mutation results in a premature termination codon (PTC) at nts 1091-1093. Downstream of the deletion, the aa sequence of the truncated mutant form of La protein (mLa, Fig. 1B, aa 192-204) differs from the native La sequence (nLa). Native human La, mutant human La, and mouse La protein can be separated by SDS-PAGE and detected by the monoclonal anti-La antibody (anti-La mab) 3B9 (Fig. 1C, lanes 1 to 3). Human native and mutant La protein can be detected and differentiated from mouse La protein with the anti-human La specific anti-La mab SW5. We find mutant human La protein in the cytoplasm of mouse 3T3 cells transfected with a vector encoding mutant La protein (Fig. 1a) while we find the majority of native human La protein in the nucleus of 3T3 cells transfected with a vector encoding the native human La gene (Fig. 1b). Cells transfected with the plasmid containing the antibiotic resistance gene were not stained by the anti-La mab SW5 (data not shown).

Originally, we could not detect the mutation in the La gene when sequencing genomic DNA of the index patient (Fig. 1g). This was not unexpected, as a somatic mutation, generally present in a small proportion of cells, could not be detected by genomic DNA sequencing due to the simultaneous presence of wild type sequences from cells lacking the mutation [57,58]. To provide direct evidence for the persistence of the mutation in the patient, we developed a ligase chain reaction (LCR). LCR assays are highly specific and commonly used to detect somatic mutations in tumor cells [59,60]. The DNA sample of the patient used in the LCR (Fig. 2, lanes P) was isolated from a blood sample that was drawn twelve years after the blood sample that was used to prepare the cDNA library. We designed primers which allowed us to differentiate between 7(A)-, 8(A)-, or 9(A)-residues in the oligo(A)-region in exon 7 of the La gene. As shown in Fig. 2 (lanes P), we obtained ligation products for the 8A and the 7A primers when we analyzed a DNA sample of the index patient. Thus, a native and a mutant La gene should exist in the genome of the patient. In the case of a healthy donor control, we obtained ligation only with the primer pair specific for 8A residues (Fig. 2, lanes h). We did not find ligation products for the 7A primers in case of either the DNA sample of a healthy donor or DNA samples of age- and sex-matched anti-La negative SLE patients (Fig. 2, lanes h,S1,S2). No non-specific primer ligation in the absence of any DNA sample (Fig. 2, lanes N) was detected. After immunoprecipitation of total extracts of PBMCs of the index patient or that of a healthy donor using an anti-La immunoaffinity-matrix the immunoaffinity-isolated La proteins were separated by SDS-PAGE and blotted against the anti-La mab SW5. We detected a fragment consistent with the molecular weight of mutant human La in the immunoaffinity-purified sample of the index patient (data not shown).

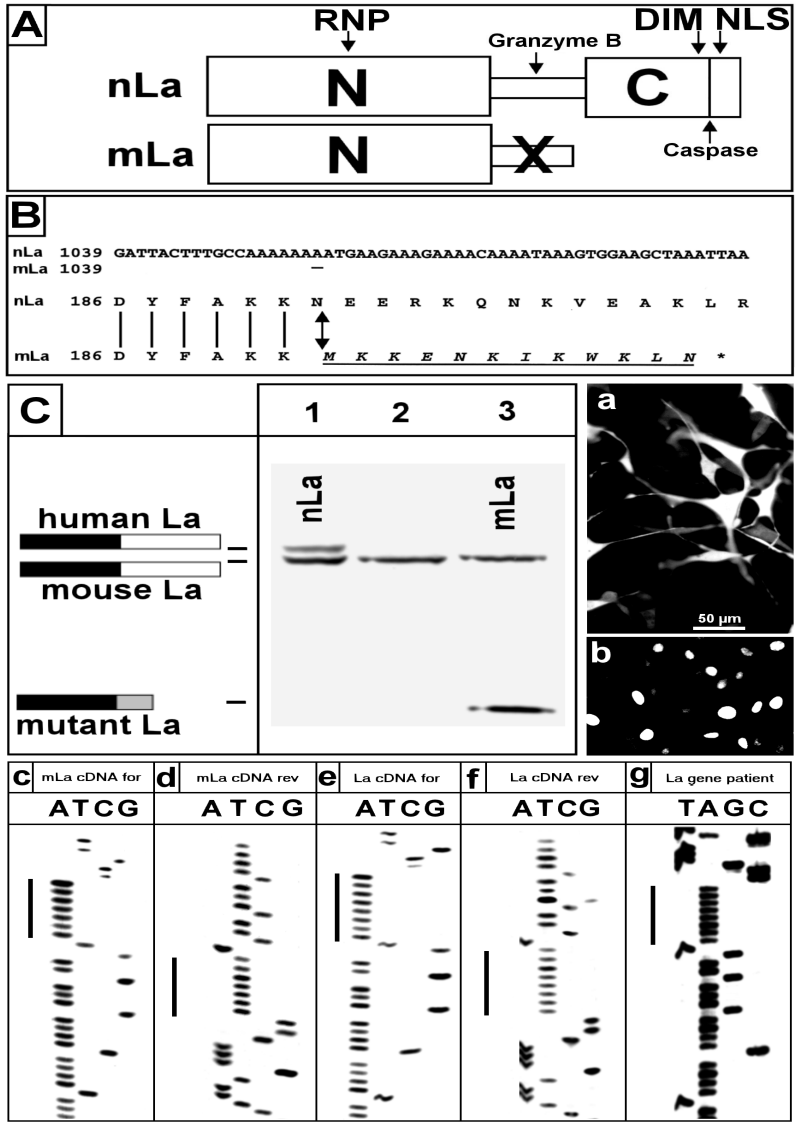

Fig. 1: Native and mutant human La protein. (A,B) native human La (nLa) consists of two domains. The N-domain contains a RNP consensus motif. The C-domain contains a nuclear localization signal (NLS) and a dimerization site (DIM). The NLS can be removed by caspases during apoptosis. The peptide connecting the N- and C-domain is sensitive for proteolysis by granzyme B. Mutant La mRNA (mLa) contains a frame shift mutation (a, X; b, - at nt position 1057) in the exon 7 encoding the peptide linking the N- and C-domain. The mutation leads to a PTC (B, *) and a truncated mutant form of La (mLa). Downstream of the frame shift mutation (B, double headed arrow) the aa sequence differs from the native aa sequence (nLa). The mutant aa sequence is underlined. (C) Total extracts of mouse 3T3 (lanes 1 to 3) were analyzed by SDS-PAGE immunoblotting using the anti-human and anti-mouse La specific antibody 3B9.

The cells were transfected with either native (lane 1) or mutant (lane 3) human La. A total extract of cells transfected with a vector control was run in lane 2. (a,b) Expression of native and mutant human La in mouse cells. Mouse 3T3 cells were transfected with genomic human La constructs designed to express either native (a) or mutant (b) human La. The cells were stained with the anti-human La specific mab SW5. (c to f) La cDNAs isolated from the index patient were sequenced. One cDNA (c,d,) had a deletion of an A residue in an oligo(A) region. Instead of 8 (A)-residues (e,f) the mutant La cDNA contained 7 (A)-residues (c,d). The mutation could not be detected by sequencing of genomic DNA of the patient (g).

Fig. 2: The mutation in the La gene of the patient persists for more than a decade. DNA of a healthy donor (h) and the index patient (P) were analyzed by a LCR assay. The DNA of the index patient was collected 12 years after the sample which was used to prepare the cDNA library and in which the mutant La cDNA was detected. The hot spot region of the La gene was amplified by PCR. In the absence of any PCR product neither the 7A nor the 8A nor the 9A LCR primers were ligated (N). The DNA of the healthy donor (h) and the index patient (P) allowed a ligation of the 8A primer. The 9A primers were not ligated in the presence of DNA of either the healthy donor or the patient DNA. The 7A primer were also not ligated in the presence of the healthy donor or two anti-La negative SLE patients (S1,S2). However, they were ligated in the presence of the index patient's DNA.

Escape of mutant La mRNA from RNA surveillance

Originally, we followed the idea that the frame shift mutation in the La gene of the patient perhaps encodes a neoepitope which initiates an immune response. Epitope spreading may lead to an autoimmune response to the native antigen. However, as schematically summarized in Fig. 3, a mutant mRNA encoding a truncated protein should be recognized by RNA surveillance mechanisms and eliminated [61-63]. According to this modell, certain marker proteins remain associated after splicing at the exon junctions. During the first round of translation these exon junction complexes (Fig. 3, EJC) will be removed by the scanning ribosome as the stop codon usually locates in the last 3'-exon. In case of a premature stop codon (PTC), the ribosome will dissociate before reaching the last exon.

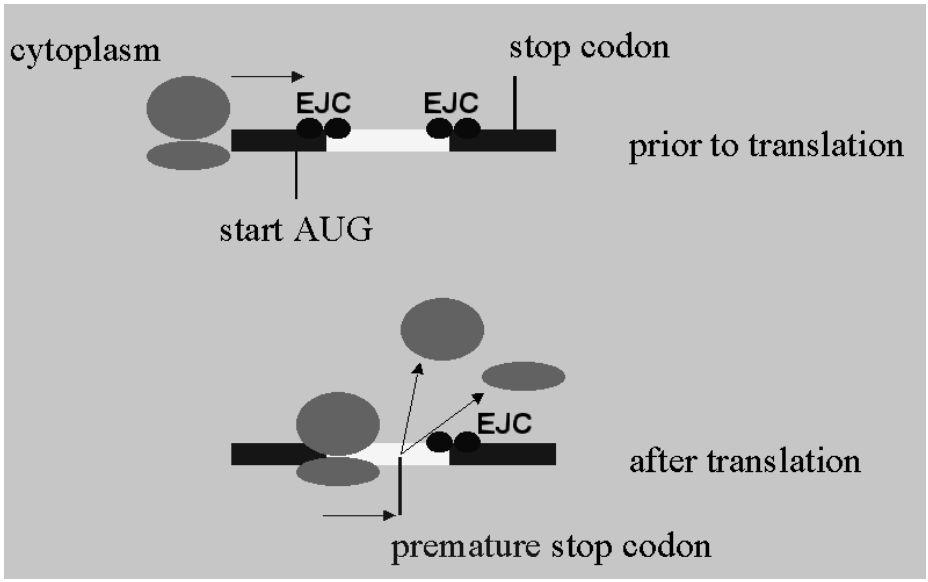

Fig. 3: RNA control mechanism. According to the 5'-scanning mechanism, prior to initiation of translation the ribosome binds to the 5'-cap structure and passes along the mRNA until it reaches the first AUG. Then the protein translation starts until the ribosome reaches the first in frame stop codon. Usually, the stop codon locates in the last exon. Thus, the ribosome will pass all exon splice junctions. After splicing proteins remain associated at the splice junctions (EJCs). They will be removed after the first translation round as the scanning ribosome will pass all these splice junctions. If the mRNA contains a PTC the ribosome will dissociated from the mRNA and, thus, the downstream locating EJCs will remain associated. These complexes can be reognized by RNA control mechanism. Then, the respective mRNA will be degraded.

In order to learn whether or not the mutant La mRNA has the stop codon (PTC), the ribosome will dissociate before reaching the last exon. Thus, it won't pass all exon junctions and, consequently, a portion of the ECJs will remain associated with the mRNA. These remaining ECJs can be recognized by RNA control mechanisms, and the identified mRNA will be degraded. However, examples from at least two genetic diseases indicate that NMD is not perfect. Rare exceptions of the rule include mutations that introduce PTCs into the human ROR2 mRNA which causes brachydaktyly type B [64] and mutations of the β-globin gene, which cause β-thalassaemias [65].

In order to learn whether or not the mutant La mRNA has the capability to escape this RNA control mechanism, two independent transgenic mouse lines (M1 and M2) were established from a mutant La gene construct. Real time PCR assays were developed and evaluated allowing us to determine the copy numbers of human and mouse La mRNAs per cell. The assays were applied to tissues from the human mutant La-transgenic, native La-transgenic and FVB/N control mice. We estimated the copy numbers of human La mRNA isoforms exon 1 and 1' in either mutant La-transgenic mice or native La-

transgenic mice or non-transgenic mice. Examples are shown in Fig. 4. We found no dramatic differences in expression of the human native and mutant La transgenes. Thus, mutant La mRNA escapes RNA surveillance.

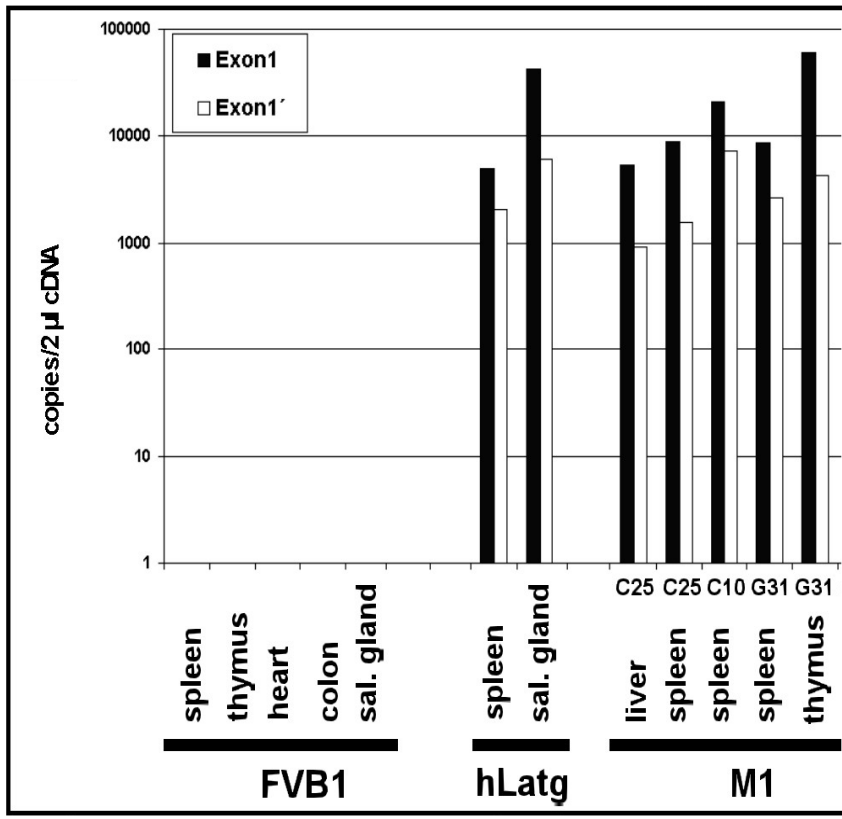

Fig. 4: Quantitative estimation of human and mouse La mRNAs in transgenic and non-transgenic mice. Six functional La mRNA isoforms can be transcribed from the human and mouse La genes [Bachmann, to be published]. The copy numbers shown represent the human exon 1 (black bars) and the human exon 1'. La mRNAs (white bars) in native human La (e.g. hLatg), mutant human La (M1) transgenic mice or non-transgenic FVB/N mice (e.g. FVB1).

We quantified the expression of the endogenous mouse La mRNAs in the absence or presence of either the native or mutant human La transgenes. The expression of the endogenous mouse La gene was not downregulated by the presence of the respective human La transgene (data not shown). Thus, the respective La transgene is expressed in addition to the endogenous mouse La gene.

In agreement with the RNA data, both mutant La-transgenic mouse lines also expressed mutant La protein in all organs tested including in spleen, liver and kidney (Fig. 5).

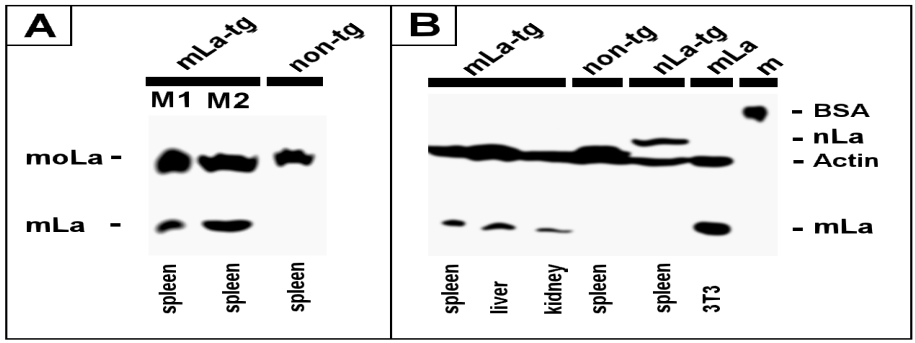

Fig. 5 (A,B): Expression of mutant La protein in transgenic mice. We prepared extracts from a cell line expressing mutant La protein and tissues from non-transgenic mice (non-tg) or from mice transgenic for either mutant La (mLa-tg) or native La (nLa-tg). The extracts were analyzed by SDS-PAGE/immunoblotting. The blots were either developed using the anti-La serum of the index patient (A) or the anti-human La specific antibody SW5 (B). The extracts were normalized using an anti-actin antibody (B). Marker proteins (m). moLa, mouse La. mLa, mutant La.

Systemic autoimmunity in mice transgenic for mutant human La

58 serum samples taken from 42 mice were analyzed by ELISA for anti-nuclear antibodies. 40 out of 42 serum samples taken from 38 animals older than 5 months contained autoantibodies to nuclear antigens (e.g. Fig. 6).

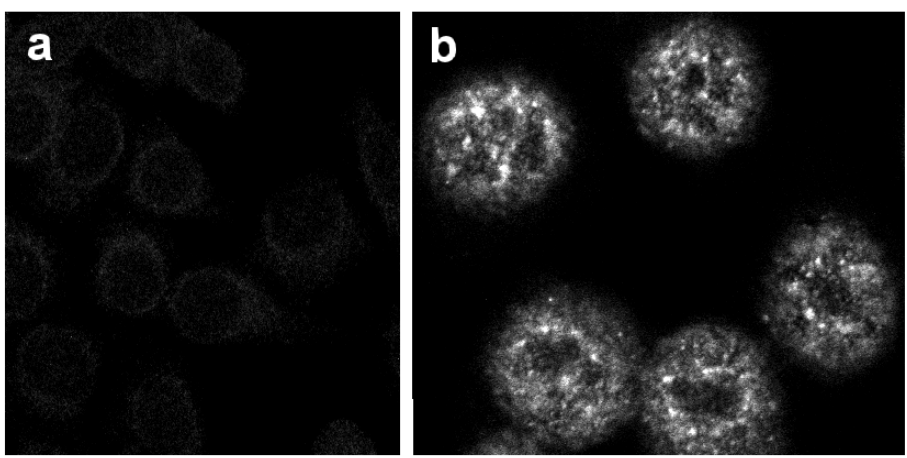

Fig. 6: Detection of autoantibodies in mutant La transgenic mice by epifluorescence microscopy.

All animals (n=18) older than eight months contained autoantibodies. The majority of mice younger than four months (n=16) and older than eight weeks had no autoantibodies or very low titers. In general, the autoantibody

response varied and development of autoantibodies did not depend on homozygosity. In addition, to autoantibodies we found evidence for immune complex deposits in tissues, and collected preliminary data supporting the occurrence of various cytopenias (data not shown).

A link between p21, Ro60, and mutant La?

As summarized above, upregulation of p21 is thought to cause a cellular senescence like phenotype of memory B/T cells which are arrested in G0/G1-phase of the cell cycle, resistant to proliferation and apoptosis, and produce large amounts of proinflammatory cytokines [43,66]. The molecular mechanism for upregulation of p21 is unclear. One transcriptional regulator of p21 is the suppressor oncogene p53 [67]. p53 itself is regulated by ubiquitinylation and sequestration mediated by the mdm2 protein [e.g. 68,69]. The mdm2 mRNA contains an internal ribosomal entry site (IRES) and translation of mdm2 mRNA requires binding of a dimer of La autoantigen to the IRES element [28,70]. Thus, the mutant La protein lacking the dimerization site could cause a dominant negative effect on translation of the mdm2 mRNA (Fig. 7).

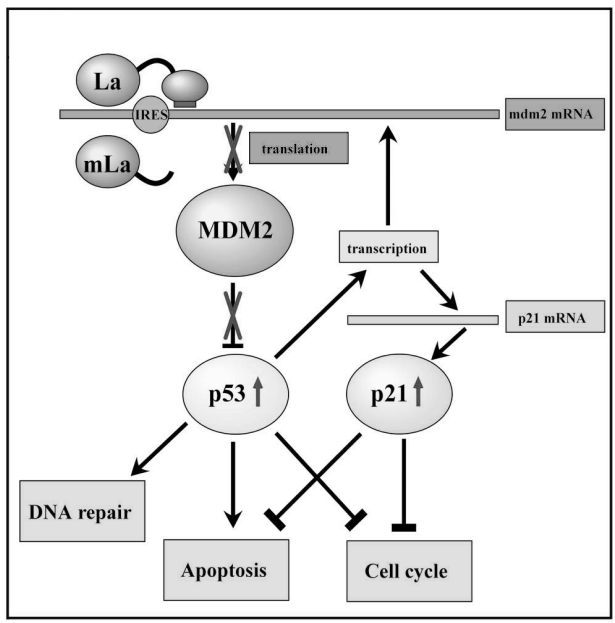

Fig. 7: A model for dysregulation of mdm2 mRNA translation by mutant La. IRES dependent translation requires dimerization. Thus, translation of mdm2 should be impaired in cells expressing mutant La as mutant La lacks the dimerization signal. Therefore, we hypothesize that mdm2 protein is less efficiently made in cells expressing mutant La. Less mdm2 results in a stabilization of p53 as mdm2 protein is

a ubiqutin ligase regulating the stability of p53. Among the direct consequences on apoptosis, DNA repair and the cell cycle, a stabilization of p53 will also result in an increase of p21 mRNA as p53 works as a transcription factor of p21.

The resulting stabilization of p53 would increase the level of p21 mRNA. Interestingly, p53 is upregulated in T cells of lupus patients in a way that correlates with disease activity [71]. Moreover, there is a clear relationship between ultraviolet radiation and the clinical manifestations of patients with SLE [72]. It is also well known that estrogens can exacerbate lupus [73]. Both, UV-irradiation and estrogens affect the expression of the mdm2 gene and binding of the estrogen receptor α to p53 protects p53 against degradation mediated by mdm2 [74,75].

In order to proof this hypothesis, we compared the expression of p21 in native La-transgenic, mutant La transgenic and non-transgenic mice. As shown in Fig. 8, p21 was clearly upregulated in all analyzed tissues including liver, kidney, spleen, heart, and thymus.

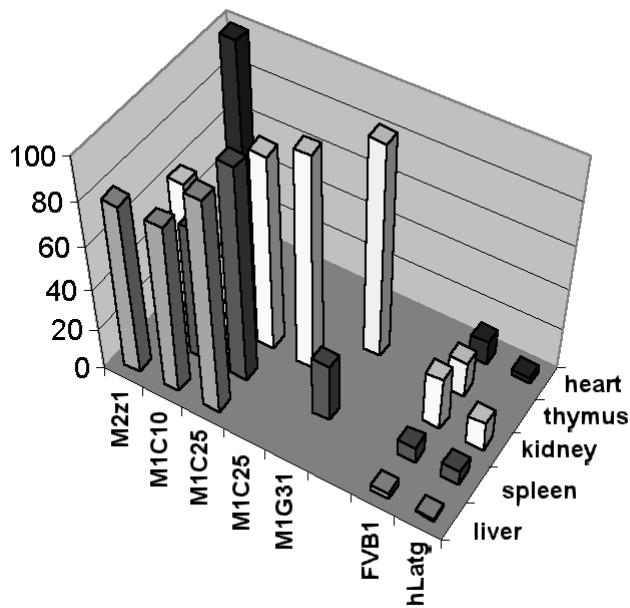

Fig. 8: Upregulation of p21 expression as a consequence of mutant La expression. p21 mRNA levels were estimated by realtime PCR for different tissues from different mutant La-transgenic mice of the two lines M1 and M2, a native human La-transgenic (hLatg) and a control FVB/N (FVB1) mouse. The absolute values varied between different tissues. Therefore, we set the highest expression in the respective tissue as 100%. We observed an upregulation of p21 in mutant La-transgenic mice but not in native human La-transgenic mice and FVB/N controls.