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Differential Responses to Smith D Autoantigen by Mice with *HLA-DR* and *HLA-DQ* Transgenes: Dominant Responses by *HLA-DR3* Transgenic Mice with Diversification of Autoantibodies to Small Nuclear Ribonucleoprotein, Double-Stranded DNA, and Nuclear Antigens

Chao Jiang,*,† Umesh S. Deshmukh,‡ Felicia Gaskin,§ Harini Bagavant,‡ Julie Hanson,¶ Chella S. David,¶ and Shu Man Fu*,†,‡

Anti-Smith (Sm) D autoantibodies are specific for systemic lupus erythematosus. In this investigation, the influence of *HLA-D* genes on immune responses to SmD was investigated. Mice with *HLA-DR3*, *HLA-DR4*, *HLA-DQ0601*, *HLA-DQ0604*, or *HLA-DQ8* transgenes were immunized with recombinant SmD1, and their Ab responses were analyzed. Analysis by ELISA showed that all strains responded well to SmD. However, when synthetic SmD peptides were used as substrate, DR3 mice had the highest Ab response followed by DQ8, DQ0604, DQ0601, and DR4. A similar trend was observed in Western blot analysis using WEHI 7.1 cell lysate as the substrate, with the exception that DR4 mice did not generate detectable amounts of Abs. Only sera from DR3 and DQ0604 mice immunoprecipitated A-ribonucleoprotein (RNP), SmB, and SmD. Intermolecular epitope spreading to A-RNP and SmB was evident in DR3 and DQ0604 mice, as sera depleted of anti-SmD Abs were reactive with these proteins. DR3 mice also generated an immune response to C-RNP. Anti-nuclear Abs were detected in the majority of the DR3 mice, whereas moderate reactivities were seen in DQ0604 and DQ8 mice. Interestingly, only DR3 mice mounted an anti-dsDNA Ab response. Approximately half of the anti-dsDNA Abs were cross-reactive with SmD. Ab responses correlated with the strength of the T cell responses. Thus, *HLA-DR3* appears to be the dominant *HLA-D* gene that determines the magnitude and quality of the anti-SmD immune response. In addition, our findings provide insights into the origin of the anti-dsDNA Abs often detected in patients with systemic lupus erythematosus. ***The Journal of Immunology*****, 2010, 184: 1085–1091.**

Systemic lupus erythematosus (SLE) is a multisystemic disorder with protean clinical presentations. The disease is characterized by the presence of autoantibodies with diverse specificities. Among the autoantibodies, anti-Smith (Sm) Abs have been considered more specific for SLE (1). Recent evidence suggests that the generation of these lupus-related autoantibodies is Ag-driven and depends on T cell responses to these Ags. This conclusion is further supported by the genetic finding that *HLA-DR2* and *HLA-DR3* are the major susceptibility genes in the pathogenesis of SLE (2–4). In addition, a study from multiplex families has shown that responses of anti-Sm Abs are linked to *HLA-DR3* homozygosity (2). Therefore, it is of interest to study the role of *HLA-DR3* in the generation of anti-Sm Abs.

Although many studies have been reported regarding levels of various autoantibodies in patients with SLE and the relationship to the HLA complex (5), it is difficult to design a study to determine the roles of a specific *HLA-D* gene in either healthy individuals or in patients. This difficulty is applicable to other autoimmune disorders. To circumvent this difficulty, humanized mice, which express human HLA class II Ags, have been used. These transgenic mice have been informative as animal models for human autoimmune diseases (6, 7). In addition, mapping T cell epitopes of many autoantigens has been accomplished using these mice. Some examples are the mapping of T cell epitopes of collagen in collagen-induced arthritis (8), preproinsulin and proinsulin in diabetes mellitus (9), proteolipid protein in experimental autoimmune encephalitis (10), retinal soluble Ag in experimental autoimmune uveitis (11), Ro60 (12), and La (13) in SLE.

In this investigation, several HLA-D transgenic mouse strains were used to study the role of HLA-D Ags in immune responses to SmD following immunization with recombinant SmD molecule. The data support the conclusion that *DR3* is the dominant gene in determining the magnitude and diversity of the response to SmD. In addition, the anti-SmD response may initiate the production of the anti-dsDNA Abs, an autoantibody specificity that is thought to be of clinical significance.

Materials and Methods

Synthetic peptides and recombinant SmD1 protein

A set of synthetic overlapping peptides covering the whole SmD protein (1–119) was obtained from the Biomolecular Research Core facility of the University of Virginia (Charlottesville, VA). The peptides were 15 aa long

*Division of Rheumatology and Immunology, Department of Medicine; †Department of Microbiology, ‡Division of Nephrology and Center for Immunity, Inflammation and Regenerative Medicine, Department of Medicine, and §Department of Psychiatry and Neurobehavioral Sciences, University of Virginia School of Medicine, Charlottesville, VA 22908; and ¶Department of Immunology, Mayo Clinic, Rochester, MN 55905

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Address correspondence and reprint requests to Dr. Shu Man Fu, University of Virginia Health System, Box 800133, Charlottesville, VA 22908-0133. E-mail address: sf2e@virginia.edu

Abbreviations used in this paper: ANA, anti-nuclear Ab; LNC, lymph node cell; RNP, ribonucleoprotein; SI, stimulation index; SLE, systemic lupus erythematosus; Sm, Smith; snRNP, small nuclear ribonucleoprotein.

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with an overlap of 12 aa over the previous peptide. Although the length of the peptides could have been in the range of 12–20 aa, the choice of the 15 mers was made on the basis that 15 mers in general give optimal binding to class II molecules and TCRs. This was confirmed using MHC class II binding algorithm (www.syfpeithi.de), wherein the core nonamer sequence is flanked by 3 *N*-terminal aa and 3 C-terminal residues. Cloning, expression, and purification of 6X His-tagged recombinant SmD protein has been described before (14).

Mice and immunizations

All experiments performed on mice were approved by the Institutional Animal Care and Use Committee. The following HLA transgenic mice were used in this study: Aβ0DR3 (*DRB1∗0301*), Aβ0DQ0601 (*DQA1∗0103/DQB1∗0601*), Aβ-0DQ0604 (*DQA1∗0103/DQB1∗0604*), Aβ0DQ8 (*DQA1∗0301/DQB1∗03-02*), and Aβ0DR4 (*DRB1∗0401*). The generation and characterization of these mouse strains has been described previously (15–18). These transgenic lines express mouse CD4 and endogenous Eα and are on B10 background. For performing in vitro lymph node cell (LNC) proliferation assays, mice were immunized with 100 μg purified recombinant SmD protein emulsified in CFA in one foot pad and the base of the tail. For Ab analysis, mice were immunized similarly with SmD and followed by additional injections on days 14 and 28 with 50 μg SmD protein emulsified in IFA by i.p. route. Control mice were injected with only adjuvants. Mice were bled at different time points and sera stored at 20° C until use. Unless mentioned otherwise, data in this manuscript is from mice at 90 d after the first injection.

Ab analysis

Mouse sera were characterized for reactivity to SmD protein and SmD peptides by ELISA as described before (19). Reactivity to different cellular proteins was determined by Western blotting, using mouse WEHI7.1 cell extract as described previously (19). Reactivity to dsDNA was determined in ELISA, using plasmid DNA as substrate by a method described previously (20). To determine Ab cross-reactivity, sera were absorbed with SmD coupled sepharose beads as previously described (14). Ability of absorbed sera to react with SmD was determined by immunoprecipitation assay using ^{35}S-labeled SmD by a previously described method (19). Presence of anti-nuclear Ab (ANA) in immune sera (1:50 dilution) was determined by indirect immunofluorescence using methanol-fixed HeLa and 3T3 cell lines as substrates (21). Presence of Abs reactive with A-ribonucleoprotein (RNP), SmB, and SmD in immune sera was analyzed by immunoprecipitation assay as described previously (14). In the experiments presented in Fig. 1, Student *t* test was performed and $p < 0.05$ was considered to be significant.

LNC proliferation assay

T cell epitopes on SmD were mapped by using LNC proliferation and [^{3}H] thymidine incorporation assay as described previously (22). Draining LNCs from mice immunized with either SmD/CFA or CFA alone were cultured in the presence of synthetic peptides and SmD protein for 4 d. During the final 16 h of culture, plates were pulsed with [^{3}H]thymidine. Cells were harvested and incorporated radioactivity measured using a β plate counter. The results are expressed as stimulation index (SI), which is calculated as a ratio of mean triplicate cpm with peptide to mean triplicate cpm without peptide. An SI >2 was considered as positive response. This cutoff was based on our previous T cell epitope mapping studies for lupus-associated autoantigen Ro60 (12, 22).

Results

Anti-SmD Ab responses by HLA-D transgenic mice

Aβ^{0}DR3, Aβ^{0}DR4, Aβ^{0}DQ0601, Aβ^{0}DQ0604, and Aβ^{0}DQ8 mice on B10 background responded to immunization with recombinant SmD1 (SmD). As shown in Fig. 1, at 90 d after the initial immunization, all strains generated a good Ab response to SmD as analyzed in ELISA with SmD as the substrate. Comparison among different strains showed that DQ0601 mice gave the lowest response. It is of note that sera from DR3 mice treated with Freund's adjuvants had some reactivity to SmD in this assay.

A panel of overlapping peptides covering the whole SmD molecule was used to determine the heterogeneity of the immune responses by these five HLA-D transgenic strains of mice. As shown in Fig. 2, pooled anti-SmD sera from DR3 mice recognized the greatest number of peptides with the strongest reactions. The order of reactivities of the

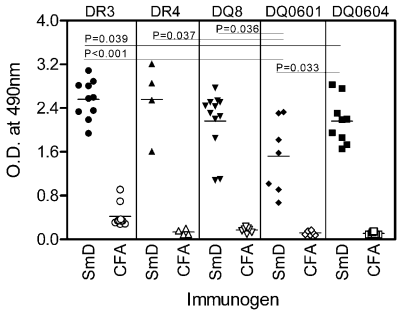

FIGURE 1. HLA-DR and HLA-DQ transgenic mice immunized with SmD protein generate a robust Ab response against the immunogen. Reactivity of sera with SmD was analyzed in ELISA. Filled symbols represent individual serum samples from transgenic mice immunized with SmD. Open symbols represent sera from mice immunized only with adjuvants. Data are shown for sera obtained 90 d after immunization, at 1:300 dilution, and are represented as mean duplicate OD at 490 nm. To determine statistical significance, Student *t* test was performed and $p < 0.05$ was considered significant. Mean OD values in all strains of mice are significantly higher than the HLA-DQ0601 mice.

pooled sera from each of the strains were DR3 > DQ8 > DQ0604 > DQ0601 > DR4. Thus, the numbers of B cell epitopes recognized by these transgenic lines vary significantly from one strain to another. These results were confirmed with another cohort of mice.

Autoantibody diversification in HLA-D transgenic mice immunized with SmD

Sera at 1:100 dilutions from SmD immunized HLA-D transgenic mice (90 d) were analyzed for their reactivities against cellular Ags in the cellular extract of WEHI 7.1 by Western blot analysis. The reactivities of three of each congenic strain are shown in Fig. 3. DR3 transgenic mice had the strongest reaction to various cellular constituents, evident by the strong bands revealed by two of the three sera (Fig. 3, *lanes 4–6*). The three immune sera from the DQ8 mice reacted with multiple bands as well (Fig. 3, *lanes 13–15*). However, the intensities were less than those seen with the DR3 immune sera. The immune sera from DQ0604 and DQ0601 were weaker than those of DR3 and DQ8 mice. Although the immune sera of DR4 mice had significant reactivity against SmD by ELISA, they hardly recognized any bands in Western blot analysis. None of the control sera from mice treated with Freund's adjuvants recognized any bands (Fig. 3, *lanes 1–3*).

Autoantibody diversification was further documented by immunoabsorption and immunoprecipitation. Pooled immune sera were used for immunoabsorption. After absorption with SmD linked to sepharose beads, only sera from DR3 and DQ0604 mice retain reactivities to several bands by Western blot analysis. The results are shown in Fig. 4. In the case of DQ0604, the absorbed sera reacted with SmB and A-RNP (Fig. 4, *left panel*). The absorption abolished the sera reactivity to SmD and several bands of higher molecular weights. The DR3 sera were more reactive than the DQ0604 sera, in that they recognized many more bands. The absorbed sera recognized SmB and A-RNP. In addition, they recognized a molecule with the mobility similar to that of C-RNP. This reactivity appears to be found only in immunized HLA-DR3 mice. The pattern of epitope spreading in response to SmD immunization is in accordance to that observed in patients' sera and to A/J mice response to SmD immunization (14).

For immunoprecipitation, only pooled immune sera from HLA-DR3 and DQ0604 transgenics were positive. Therefore, individual immune sera from HLA-DR3 and DQ0604 mice were used (Fig. 5). Many of the immune sera precipitated labeled SmB and A-RNP in addition to SmD. In general, the immune sera from DR3 mice had

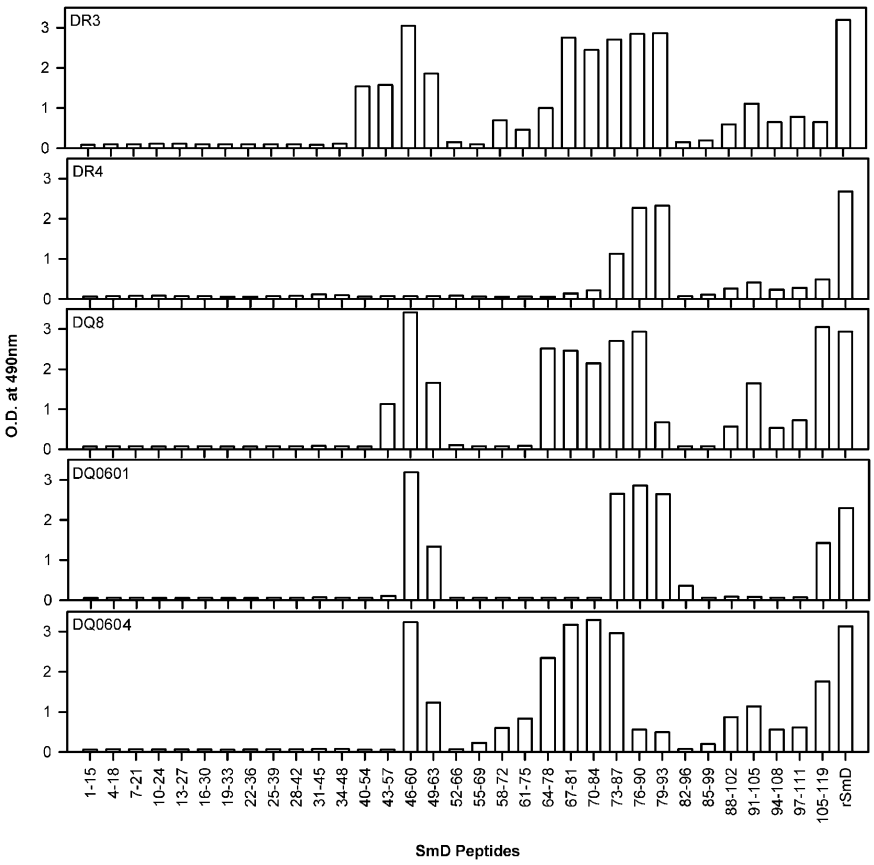

FIGURE 2. SmD B cell epitope specificities are different between the HLA-DR3 and HLA-DQ transgenic mice. Sera from mice immunized with SmD were pooled and their reactivity with overlapping peptides of SmD was analyzed in ELISA. Data are shown for sera obtained 90 d post-immunization and were used at 1:100 dilution. Sera from HLA-DR3 transgenic mice recognized more SmD peptides than any other group of mice.

stronger reactivity to these three proteins. It is of note that one of the DQ0604 sera had no reactivity, and three of them were able to precipitate SmD and/or A-RNP without apparent reactivity to SmD. This is reminiscent of the findings when SmD peptides were used as immunogens (19).

Strong ANA and anti-dsDNA Ab reactivity detected in sera from SmD immunized DR3 mice

As further evidence for autoantibody diversification, some of the SmD immune sera from DR3, DQ0604, and DQ8 stained nuclei of HeLa (Fig. 6) and 3T3 cell lines. For DR3, 6 of the 10 immune sera were strongly positive for ANA. This strong reactivity was also detected in three of the nine immune sera from DQ0604 and 3 of 12 DQ8 immune sera. One of the seven SmD immune sera from DQ0601 was weakly positive. No DR4 immune sera were positive in this assay. With two exceptions, none of the sera from Freund's adjuvant immunized mice stained the nuclei. The two reactive sera were from DR3 mice and were positive without any treatment at the initiation of the experiment.

The sera from HLA-D transgenic mice immunized with SmD were assayed for anti-dsDNA Abs by ELISA with circular plasmid DNA as the substrate. As shown in Fig. 7*A*, DR3 immune sera were found to be strongly reactive with dsDNA, whereas other immune sera and the vast majority of the Freund's adjuvant immunized sera showed little such reactivity.

Depletion of anti-dsDNA Abs in DR3 immune sera with SmD

Pooled DR3 immune sera were absorbed with solid phase SmD. As shown in Fig. 7*B*, anti-SmD Abs were absorbed completely with the solid absorbent. The absorbed sera remained active against dsDNA (Fig. 7*C*). Approximately half of the Abs were absorbed by solid phase SmD, indicating that approximately half of the anti-dsDNA was cross-reactive with SmD, the immunogen.

Strong anti-SmD T cell responses in DR3 mice

As shown in Fig. 8, DR3 mice mounted the strongest T cell proliferative response to SmD immunization. An SI >2.0 was considered as positive and was based on our previous mapping studies for Ro60 done in HLA-D transgenic (12) as well as nontransgenic A/J and SJL/J strains of mice (22). DR0604 and DQ8 mice mounted a moderate response, and DQ0601 mice mounted a weak response. DR4 transgenic mice had no discernable T cell response when they were immunized with SmD in a similar manner. Regarding T cell epitopes on SmD recognized by these HLA-D transgenic mice, more epitopes were recognized by DR3 mice. More limited numbers of such epitopes were recognized by DQ0604, DQ8 and DQ0601. It appears that the strengths of the B cell responses to SmD immunization correlate with the strengths of T cell responses of the HLA-D transgenic lines.

Discussion

In this study, several strains of mice expressing different HLA-D Ags were used to explore their responsiveness to immunization with SmD. The DR3 transgenic mice responded with high titers in the SmD ELISA assay, and their immune sera recognized the largest number of SmD peptides. In addition, the immune sera contained Abs with the most diverse specificities as revealed by Western blot analysis, and by the highest frequency of ANA. More importantly, anti-dsDNA autoantibodies were found only in the immune sera of the DR3 mice. T cells from immunized DR3 mice responded best in vitro in a recall proliferative assay. These T cells also responded to

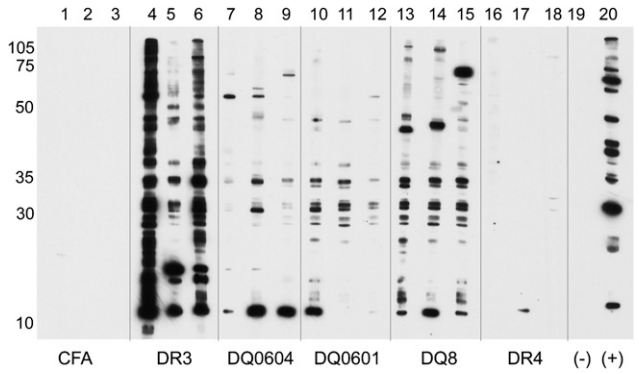

FIGURE 3. Diversification of autoantibody responses in HLA-D transgenic mice immunized with SmD. Reactivity of sera with different cellular proteins was analyzed by Western blotting using WEHI 7.1 cell extract. All sera were tested at 1:100 dilution. Reactivity of representative serum samples from different transgenic mice is shown. As control, only reactivity of sera from CFA/IFA immunized DR3 mice are shown. Sera from HLA-DR3 and HLA-DQ8 mice immunized with SmD recognize the most number of cellular proteins.

many more SmD peptides in comparison with those from other HLA-D transgenic strains. These results indicate that *DR3* is the dominant gene determining the quantity and the quality of the response. These results also agree with the observation that *HLA-DR3* is one of the major and dominant lupus susceptibility genes. Considering that mouse and human SmD have identical protein sequences, the results in this investigation support the thesis that the DR3 transgenic mouse is an excellent model for studying the pathogenesis of SLE.

In this investigation, only HLA-DR3 and HLA-DR4 transgenic mice were compared with three HLA-DQ transgenic strains. Although only two HLA-DR strains were used, significant information was generated. *HLA-DR3* and *HLA-DR4* represent two important alleles, in that *HLA-DR3* is linked to SLE (3, 4), and *HLA-DR4* is linked to rheumatoid arthritis (23). These two autoimmune disorders are the most prevalent rheumatic diseases with distinct serologic markers. Thus these findings—that only the HLA-DR3 mouse responds to recombinant SmD immunization with diverse autoantibody production and that the HLA-DR4 transgenic mouse fails to do so despite its ability to generate autoantibodies to the immunogen—add further credence to the importance of *HLA-DR3* in the initiation of lupus-related autoantibodies. In comparing the responsiveness of the HLA-DR3 transgenic mouse to that of the DQ transgenic mice and the previous findings of HLA-D transgenic mouse responsiveness to Ro60 (12), it may be reasonable to conclude that HLA-DQ molecules might play a secondary role in the generation of lupus-related autoantibodies.

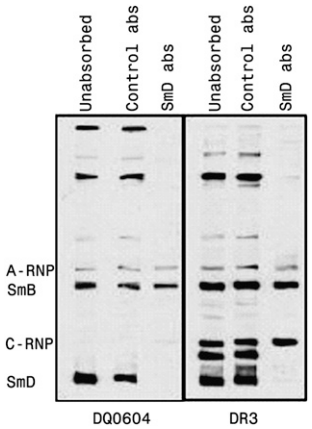

FIGURE 4. Intermolecular epitope spreading to other proteins within the snRNP complex occurs in HLA-D transgenic mice immunized with SmD. Pooled sera from HLA-DQ0604 and HLA-DR3 mice immunized with SmD were absorbed with either SmD-coupled sepharose beads or an equivalent amount of control beads. Reactivity of unabsorbed and absorbed sera with different proteins was analyzed by Western blotting. Absorption with SmD-beads completely depleted Abs reactive with SmD and some additional proteins. However, in DQ0604 mice, reactivity to A-RNP and SmB—and in DR3 mice reactivity to A-RNP, SmB, and C-RNP—persisted, which is indicative of epitope spreading.

It is highly significant that HLA-DR3 and HLA-DQ0604 mice respond well to immunization with SmD. We have previously demonstrated that mice with B6 and BALB/c background do not respond to immunization with recombinant lupus related autoantigens such as SmD (24). In contrast, SJL and A/J mice that have some autoimmune tendencies respond well with significant autoantibody diversification (24). It may be inferred from this observation that T cell epitopes presented in the context of HLA-DR3 and DQ0604 deliver a significant and strong activation signal to autoreactive T cells, resulting in the generation of autoantibodies with diverse specificities. In addition, the detection of autoantibodies to the C-RNP protein in HLA-DR3 but not in HLA-DQ0604 mice suggests that HLA-DR3/SmD peptide complexes provide a stronger signal. The patterns of autoantibody diversification in the HLA-DR3 and HLA-DQ0604 transgenic mice are similar to those observed in A/J mice (19) and in patients with SLE (2). It was also observed in this study that some of the mice despite epitope spreading to other proteins within the small nuclear ribonucleoprotein (snRNP) particle showed little response to SmD, the immunogen. This pattern of spreading was demonstrated by our previous study in A/J mice immunized with a SmD peptide, leading to the hypothesis that serologic analysis of autoimmune sera at a given time point should not be used as evidence to suggest the nature of the initiation Ags (19). The absence of anti-SmD Abs in patients' sera with autoantibodies to SmB and/or A-RNP does not imply that immune response to SmD is not occurring.

Several techniques were used in this investigation to document autoantibody diversification. It is notable that DR4 immune sera were found to be strongly reactive with SmD in ELISA, although they did not have detectable amounts of Abs to various autoantigens by Western blot analysis, immunoprecipitation, and immunofluorescence. This pattern is similar to the observation that ELISAs as clinical tests for the presence of Abs diagnostic of SLE and other autoimmune disorders are nonspecific and are of little clinical application (25). Our results add further support to the hypothesis that the current trend to replace the traditional immunoprecipitation methods with ELISAs using recombinant proteins should be evaluated.

Anti-dsDNA Abs are considered an important class of autoantibodies in SLE (26–28). These Abs are more specific for SLE, and they often correlate with disease activities. Despite intense investigations, the origin of these Abs remains controversial. The consensus is that they cannot be readily induced with purified DNA as the immunogen (29). Putterman and Diamond showed induction of anti-dsDNA Abs in normal mice through the immunization with a peptide mimic (of an anti-dsDNA monoclonal Ab) coupled to T cell epitopes (30, 31). Riemekasten et al. (32, 33) reported that peptide $SmD_{83\text{-}119}$ was able to provide T cell help for the production of anti-dsDNA Abs. In this study, NZB/NZW F1 female mice were used. However these mice have significant numbers of plaque forming cells with specificities for SmD or dsDNA. Therefore, in their system, the investigators dealt with augmentation of an ongoing response. The mechanisms for the augmentation may be multiple and were not studied further. Thus, induction of anti-dsDNA Ab production following immunization with lupus autoantigens needs to be investigated in detail.

The findings that, as a part of response to SmD immunization, DR3 transgenic mice are able to produce anti-dsDNA Abs, some of which are cross-reactive with SmD, are of considerable interest. This

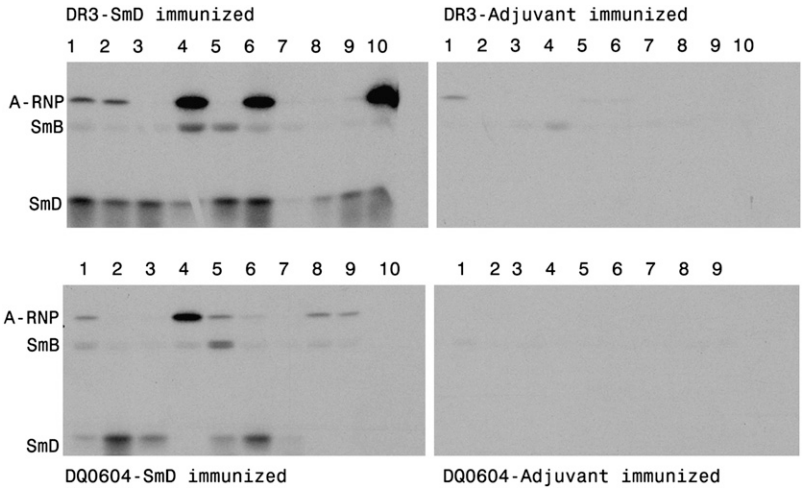

FIGURE 5. Sera from HLA-DR3 and HLA-DQ0604 transgenic mice immunoprecipitate A-RNP, SmB, and SmD. To confirm the reactivity of sera with proteins within the snRNP complex, sera were used to immunoprecipitate [^{35}S] labeled–A-RNP, -SmB, and -SmD. Sera (5 μl) obtained 3 mo postimmunization were used to immunoprecipitate the in vitro transcribed and translated proteins. A representative autoradiograph is shown.

phenomenon is both specific for DR3 and Ag dependent. In the present investigation, it is shown that DR4, DQ0601, DQ0604, and DQ8 mice did not produce such anti-dsDNA Abs. In the case of DQ0604 and DQ8 mice, they produced ANAs without the production of anti-dsDNA Abs. Although DR2 mice were not included in this study, they might be able to produce anti-dsDNA Abs as a response to SmD immunization. Our results support the hypothesis that the observed immune response is dependent on a specific *DR* gene. In a previous study, Paisansinsup et al. (12) reported that ANAs were detected in all DR2, DR3, and DQ8 mice in response to recombinant human Ro60. None of them made anti-dsDNA Abs. It is also important to stress that both the recombinant Ro60 in previous studies and the recombinant SmD in this study have the 6X-His tag. The fact that anti-DNA Abs are seen only in HLA-DR3 transgenic mice immunized with SmD indicates that the Abs cross-reactive with SmD and dsDNA are not likely to be due to an immune response to the 6X-His tag. The observation that no DNA was detected in the recombinant SmD suggests that the cross-reactivity is not due to the presence of dsDNA in the SmD. These observations together with the findings presented in this study support the hypothesis that the anti-dsDNA response is specific for SmD as the immunogen. The absorption experiments showing that approximately half of the anti-dsDNA Abs could be absorbed by solid phase SmD requires further comments. This is reminiscent of our previous finding that a significant amount of anti-SmB and anti–A-RNP Abs were cross-reactive with the immunogen SmD (19). The specific anti-SmB and anti–A-RNP Abs were generated as the Ag-specific T cells were expanded during the immune response (34). In this regard, it would be of interest to determine whether some of the induced anti-dsDNA Abs are cross-reactive with SmB, A-RNP, or C-RNP, to which the autoimmune response to SmD are diversified.

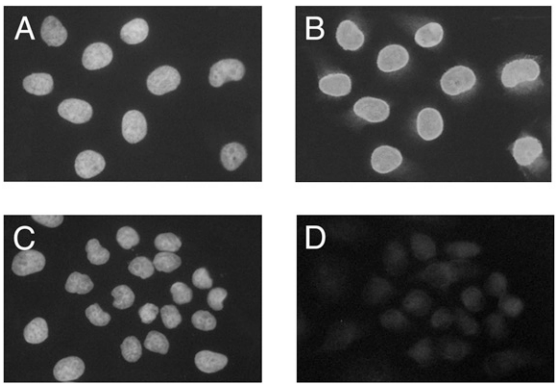

FIGURE 6. Antinuclear Abs are generated in HLA-DR3 transgenic mice immunized with SmD. Reactivity of representative serum samples from HLA-DR3 transgenic mice, immunized with either SmD (*A*, *B*) or only adjuvants (*C*, *D*) is shown. *A* and *C* show nuclei stained with DAPI. *B* shows the presence of ANA and *D* shows the absence of ANA using indirect immunofluorescence for Ab detection. The sera were used at 1:50 dilution.

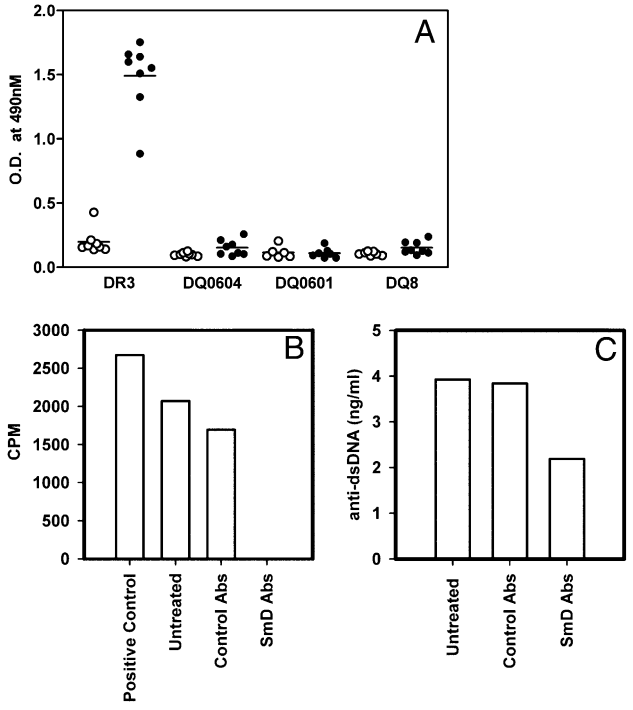

FIGURE 7. Only HLA-DR3 transgenic mice immunized with SmD generate anti-dsDNA reactive Abs. *A*, Sera from different HLA-D transgenic mice, obtained 90 d postimmunization were tested for reactivity to dsDNA. Filled symbols represent sera from SmD immunized mice, and open symbols represent sera from adjuvant immunized mice. *B*, To determine whether anti-dsDNA Abs were cross-reactive with SmD, pooled sera from DR3 transgenic mice were absorbed with SmD-coupled beads. Absorbed sera were used to immunoprecipitate ^{35}*S*-labeled SmD. The precipitated radioactivity was measured by scintillation counting and data are represented as cpm. All Abs reactive with SmD were depleted following absorption. *C*, SmD absorbed sera were tested for their reactivity to dsDNA in ELISA. Data are represented as amount of anti-dsDNA Ab (nanograms per milliliter), which was calculated using a purified anti-dsDNA monoclonal Ab as standard. Almost 50% of anti-dsDNA Ab reactivity is reduced following absorption with SmD. Control absorption (abs) was performed with untreated sepharose beads.

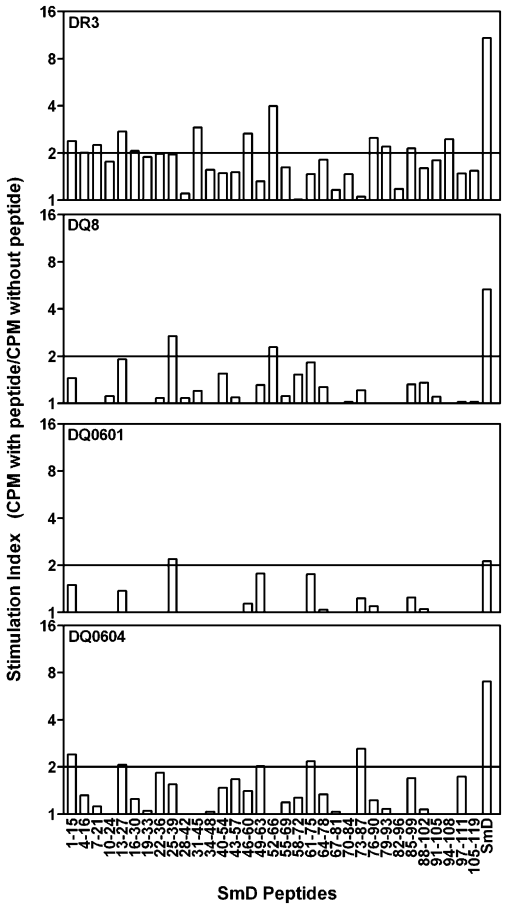

FIGURE 8. HLA-DR3 mice recognize more T cell epitopes on SmD protein. Different HLA-D transgenic mice (3–5 per group) were immunized with SmD in the foot pad and the base of the tail. On day 12, draining LNCs were used to establish a proliferation assay. Synthetic peptides were used at a final concentration of 10 μm, and reactivity to SmD is shown at a concentration of 10 μg/ml. Data are represented as SI, which is the ratio of mean triplicate cpm with peptide to mean triplicate cpm without peptide. An SI >2.0 was considered positive and is indicated by a solid line. Similar results were obtained in an additional cohort of mice.

It would also be of interest to determine whether anti-dsDNA Ab response is a part of the immune responses to SmB or A-RNP immunization. At the clinical level, it is of interest to note that anti-dsDNA Abs are often found in patients with anti-Sm Abs. Our findings might also explain this clinical observation.

From the above discussion, it appears that anti-dsDNA Abs are produced in a pattern similar to certain autoantibodies as a part of diversification of the immune response to SmD immunization. This provides evidence to support the hypothesis that anti-dsDNA is generated resulting from immune responses to protein autoantigens. This observation is also congruent with the previous observations that in MRL/*lpr* mice the monoclonal Abs against SmD Ag are often cross-reactive with dsDNA (35), and that Abs to snRNP, ribosomal P protein, and other lupus autoantigens are partly cross-reactive with dsDNA (36–38). Thus, our data support the hypothesis that our Ag-induced model for anti-dsDNA Ab production resembles that observed in both spontaneous lupus-prone mice and patients with SLE. It can also be speculated further that these Abs can be a part of the host immune responses to pathogens or other environmental Ags. This hypothesis provides an explanation that anti-dsDNA Abs are occasionally found in normal individuals without other lupus-related autoantibodies and without any clinical manifestations of SLE. It is likely that further investigations to seek support for the proposed hypothesis will settle the issue regarding the origin of anti-dsDNA Abs found in patients and healthy individuals.

Recently, it was demonstrated in our laboratory that microbial Ags can mimic SmD peptide to induce an anti-SmD response in DR3 mice (39). This observation bears testimony to the usefulness of the DR3 mice to probe the pathogenesis of SLE. The observation also adds impetus to design research to support the hypothesis that molecular mimicry among environmental Ags and autoantigens plays a major role in the initiation of autoantibody responses. Evidence in the literature suggests that autoantigens are required for autoantibody diversification (22) and that TLRs play a crucial role in the amplification of these responses (40). The availability of the HLA-D transgenic strains and well-defined lupus models together with translational research will undoubtedly provide further insight to the pathogenesis of this disorder.

Disclosures

The authors have no financial conflicts of interest.

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