An experimental IgA nephropathy induced by an environmental mycotoxin, nivalenol

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Introduction

IgA nephropathy (IgAN) was first described by Berger and Hinglais in 1968. However, no particular pathogenic cause has been elucidated in human IgAN. IgAN is characterized by its features: IgA is predominantly deposited in glomeruli with various grades of mesangial proliferation in every patient, and serum IgA levels are elevated in nearly half of IgAN patients. It is also known that IgA specific regulatory lymphocytes are well represented in the mucosal immune system and IgA production is initiated primarily in this system. In light of these facts, I hypothesized that IgAN is triggered by some exogenous antigen(s) which induces dysregulation of the mucosal immune system. One more remarkable feature of IgAN is that the apparent incidence of this disease varies greatly from country to country. The disease is particularly prevalent in southern Europe, Southeast Asia, and Australia. In Japan, IgAN is the most common form of glomerulonephritis and the incidence of this disorder amounts to about 30 to 40 percent of patients with primary glomerulonephritis who underwent renal biopsy.

In 1989, Pestka and colleagues reported that an environmental mycotoxin, deoxynivalenol (DON), induces some pathological changes in mice which resemble human IgAN. It has been pointed out, however, that common agricultural products such as corn and oats were more commonly contaminated with nivalenol (NIV) than with DON in Southeast Asia and Japan where IgAN is more frequently found. NIV (3, 4, 7, 15-tetrahydroxy-12, 13-epoxy trichothecene 9-en-8-on) was first isolated in Japan from the metabolites of Fusarium nivale Fn 2B as a causal agent of scabby wheat intoxication in 1968.

Thus, based on the mucosal immunity-oriented hypothesis of IgAN and the more frequent occurrence of this disease in Southeast Asia and Japan as described above, I thought it meaningful to determine whether orally-ingested NIV can, in fact, induce pathological changes in glomeruli of mice, which resemble those in human IgAN. It was further examined whether immunological alterations of lymphocytes isolated from Peyer's patches (PP), an important IgA inductive site or gut-associated lymphoid tissues (GALT), occurred in the NIV-induced IgAN after we established the NIV model.

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Experimental Design

Six- to eight-week-old female C3H/HeN and C3H/HeJ mice were obtained from CLEA Japan (Tokyo, Japan). For NIV administration, mice were fed semi-synthetic diets containing 6 or 12 ppm NIV for 4 or 8 weeks. Control mice were fed on the same semi-synthetic diets without NIV. All mice were given these diets ad lib. For comparison, a part of mice were orally given ferritin (Sigma Chemical Co., St. Louis, MO) at 10 mg in 0.2 ml by gastric intubation on the first day, followed by 1 mg/ml of drinking water for 4 wk by the modified method of Genin and colleagues.

After completion of the oral presentations, the levels of IgG, IgM and IgA in the sera were determined by enzyme-linked immunosorbent assay (ELISA). Sections of renal cortex were examined by immunofluorescence for glomerular immunoglobulin and C3 deposition, and by light and electron microscopy for histopathological changes.

Once it was established that orally administered NIV induced IgAN-like changes, a competitive ELISA was carried out to confirm whether or not the IgA antibody from the model mice truly has affinity to NIV. Adoptive transfer of PPL from the standard model mice were also carried out using six- to eight-week-old normal syngeneic mice as recipients. Moreover, an enzyme-linked immunospot (ELISPOT) assay was performed to evaluate the number of antibody-producing cells in the standard NIV model. RT (reverse transcription)-PCR (polymerase chain reaction) analysis was performed as well to detect cytokine-specific mRNA expressions of CD4+ T cells isolated from PP in the model mice. In all of these immunological studies, C3H/HeN mice fed NIV 12 ppm for 8 weeks were used as the model mice.

Differences between different study groups of mice were analyzed by Student's t-test or by Mann-Whitney U test for nonparametric analyses.

Establishment of the NIV Model

Oral administration of NIV in low doses reproducibly induced significant IgA deposits in glomerular mesangium and elevated serum IgA levels in C3H/HeN mice (Fig. 1 and 2). Mesangial IgA deposition was the most intense and serum IgA level was the most highly elevated in mice given NIV 12 ppm for 8 weeks. These changes were confirmed in mice irrespective of the strain. Namely, oral administration of NIV 12 ppm for 8 weeks also caused both significant IgA deposits (Grade 2 to 3) in the glomeruli and serum IgA elevation of BALB/c as well as C3H mice, findings not seen in those of controls (data not shown). Electron microscopy revealed mesangial expansion and numerous electron dense deposits, samll and large, scattered in the mesangial and paramesangial areas of glomeruli in NIV-fed mice (data not shown). The degree of these immuno-pathological changes analogous to human IgAN was associated with the dose and duration of NIV presentation. Oral administration of ferritin did not induce significant IgAN-like changes, different from the results previously reported by Genin and colleagues. Furthermore, a competitive ELISA with a NIV analogue-protein conjugate disclosed that the IgA antibody in sera from the NIV model mice had a higher affinity to the mycotoxin (Fig. 3). Conclusively, these findings suggest that NIV induces some pathological changes in mice which
Fig. 1  Intensity of IgA deposits in the mesangium. The intensity of immuno-fluorescence for IgA in each group was graded from 0 to 3; 0 = no deposit, 1 = weakly stained, 2 = moderately stained, 3 = strongly stained. Each column shows percentage of kidney samples belonging to each grade. Each group included nine to twelve mice.

Fig. 2  Serum IgA level in mice presented with NIV and controls. NIV was presented at two different doses of 6 ppm and 12 ppm, for periods of 4 and 8 weeks. Results are expressed as mean±SEM. *P<0.05  **P<0.001 (compared with respective control),  †P<0.001 (compared between mice administered ferritin and NIV 12 ppm for 8 wk).
Fig. 3 Competitive ELISA to detect NIV-specific IgA antibody. The affinity to NIV of a control serum (●) and sera from two mice fed NIV 12 ppm for 8 weeks (▲, ▼) was evaluated by optical densities at 492 nm. The affinity to NIV of those from the NIV fed mice was also evaluated in the presence of BSA (△, ▽). IgA antibodies from NIV-treated mice showed a high affinity to DON-hemi-succinate-OVA conjugate which is similar to NIV conjugate. When the IgA antibodies from the model mice were incubated with appropriately diluted NIV, the affinity to the conjugate was competitively absorbed.

resemble those in human IgAN, and that this mycotoxin is associated with the pathogenesis in some types of glomerulonephritis.

Examination of Immunological Abnormalities in GALT of the NIV Model

To examine whether lymphocytes of PP, an example of the major IgA inductive sites in GALT, are involved in the NIV-induced IgAN, an adoptive transfer experiment was first carried out using PPL isolated from mice with NIV-induced IgAN. Fifteen million of the PPL were aseptically injected intraperitoneally (i.p.) into each naive syngeneic recipient mouse. Consequently, elevation of serum IgA levels and mild mesangial IgA deposition were induced in the recipients. Adoptive transfer of control PPL isolated from normal mice did not significantly induce the same changes in the recipients.

After the transfer experiments, ELISPOT procedures were performed to more thoroughly examine the dysregulated immunoglobulin production of PPL in the NIV model. Namely, the number of IgA, IgG and IgM-producing cells were quantitated utilizing ELISPOT assay. Nitrocellulose bottom plates (Millititer HA, Millipore Corp., Bedford, MA) were coated with goat anti-mouse Ig diluted in PBS (100 µl/well). After incubation of PPL in the plates, final results were obtained with 1 × 10⁵ feeding cells/well for IgG, IgM and IgA producing spots. The number of Ig-producing cells was also evaluated as well in spleen lymphocytes (SPL) isolated from the NIV model mice. Consequently, significant increase of IgA-producing cells was confirmed with an ELISPOT procedure in PPL from the model mice (Fig. 4).

For the detection of IL-2, IL-4, IL-5, IL-6, IL-10, TGF-β and IFN-γ specific mRNA in CD4⁺ T cells isolated from PP of the NIV model mice, a modified standard RT-PCR amplification protocol was employed. Details of this procedure followed the method previously reported by Hiroi and colleagues. As a result, upregulation of CD4⁺ T cells was also confirmed in PP of
Fig. 4  Frequencies of IgA-producing cells in SPL and PPL from the NIV model and controls. Regular diets or those containing NIV 12 ppm were orally administered to mice for 8 weeks. After the administration period, cells were examined for the number of IgA spot forming cells in controls (□) and in the NIV model (■) by ELISPOT assay. Results were presented as the mean±SEM. *P<0.05.

Fig. 5  Analysis of cytokine mRNA expressions in CD4+ T cells from Peyer's patches (PP) and spleens (SP) by RT-PCR. Controls were obtained from mice fed regular diets. Experimental cells were obtained from mice fed NIV 12 ppm for 8 weeks. Each left lane shows mRNA expression in controls. Each right lane shows mRNA expression in experimental cells. ST:X174 RF DNA/Hae III fragment ladder. 1: β-actin (349 bp) 2: IFN-γ (460 bp) 3: IL-2 (502 bp) 4: IL-4 (399 bp) 5: IL-5 (243 bp) 6: IL-6 (155 bp) 7: IL-10 (455 bp) 8: TGF-β (525 bp)

Given all of these facts above, it is suggested that PPL are immunologically dysregulated in the NIV-induced IgAN, and that this kind of upregulation of the mucosal immune system may be associated with the pathogenesis of IgAN.
Conclusion

A reproducible IgAN model has been established with oral administration of low-dose NIV. This finding indicates that NIV must be considered as a possible causative agent in human IgAN. Moreover, it is reasonable to hypothesize that IgAN is triggered by some exogenous antigen(s) which induces dysregulation of the mucosal immune system, according to the present results. It was also demonstrated that lymphocytes of the PP, the immunologically major working force and inductive site in GALT, were dysregulated in this NIV model.

Acknowledgements I would like to thank Prof. Ueno for providing me the oppoutunity to introduce my research work at the 44th meeting of the Japanese Association of Mycotoxicology. This work was partly supported by Grants-in-Aid for Science Research from the Ministry of Education, Science and Culture of Japan (No. 05807100 and 07671271), and grants from the Ministry of Social Welfare of Japan, Toranomon Hospital, Okinaka Memorial Institute for Medical Research and The Shimabara Science Promotion Foundation. F. Hinoshita is a recipient of the 1993 Fellowship from the Study Group on IgA Nephropathy which is financially supported by Sumitomo Pharmaceutical Co. Ltd.

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