Assessment of Th1, Th2, and Th17 Cells in Birdshot Retinochoroidopathy

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INTRODUCTION

Birdshot retinochoroidopathy (BSRC) is a chronic inflammatory disease that appears to be isolated to the eyes, causing distinct bilateral chorioretinal lesions, and progressive vision loss which can be treated with systemic immunomodulatory therapy (IMT). The pathogenesis has not been elucidated, but lymphocytic infiltration has been found on BSRC histopathology, and the role of retinal autoimmunity has been suggested. T helper (TH)-17 cells, a subset implicated in BSRC, have recently been implicated in BSRC. Kuiper et al. showed that IL-1β, IL-6, IL-17, and tumor necrosis factor (TNF-α) were elevated in the aqueous fluid of BSRC eyes. Furthermore, IL-1β, IL-17, and TNFα were more concentrated in the aqueous than in the serum. In another study, Monnet et al. showed that interferon (IFN)-γ was elevated in the serum, but IL-17 and IL-23 were not. In this study, we will correlate the serum levels of 20 cytokines from the Th1, Th2, and Th17 pathways with disease activity and IMT in BSRC patients.

METHODS

Single center cohort study of 15 BSRC patients and 12 controls. The diagnosis of BSRC was based on the research criteria of an international consensus conference, and HLA-A29 positivity. Disease activity and IMT status, specifically CellCept and cyclosporin A, were recorded for each BSRC patient, and placed into the following subgroups: active disease naïve to IMT (n=3), active disease on IMT (n=3), remission on IMT (n=3), and remission off IMT (n=3). Disease activity was a clinical decision based on visual acuity, visual field, fundus exam, optical coherence tomography, fluorescein angiography, and electroretinography. Patients with other autoimmune disease, uncontrolled systemic disease, and recent surgery or infection within 1 month were excluded. Sera were collected and frozen until analysis. Heterophilic immunoglobulins were removed with protein L-coated agarose beads. Quantitative multiplex sandwich ELISA-based microarray assays were used to quantify 20 cytokines: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17, IL-17F, IL-21, IL-22, IL-23, IL-28, IFNγ, macrophage inflammatory protein (MIP)-3α, transforming growth factor (TGF)-β1, TNFα, TNFβ, and granulocyte macrophage colony-stimulating factor (GM-CSF). Cytokine levels from BSRC patients were compared with controls and correlated with IMT and disease activity. Two-tailed T tests with a P value of 0.05 for significance was used.

RESULTS

Serum IL-2, IL-21, IL-22, IL-23, and TGF-β1 were detectable in all active BSRC patients naïve to IMT as compared to a fraction of controls (figure 1). The percentage of detectable levels trended down with the use of IMT to nearly 0% for all cytokines in the remission on IMT subgroup. The serum level of IL-23 was significantly elevated in active BSRC patients naïve to IMT compared with controls (figure 2, asterisk; p=0.015). The significance of IL-21 and TGF-β1 levels was limited by n size and variability, but the scatter plot (figure 2 right panel) reveals a potential trend towards elevated levels. The serum levels of IL-2, IL-17, IL-22, as well as TNFα and INFγ (not shown) were not significantly different from control levels.

DISCUSSION

While Kuiper et al. showed that IL-17 levels were elevated in the aqueous fluid of BSRC eyes, Monnet et al. showed that IL-17 was not elevated in BSRC serum, which is confirmed by our results. However, contrary to Monnet et al., we found no elevation in INFγ levels, and instead found IL-23 significantly elevated in active BSRC naïve to IMT. The differences in findings may be due to our subgrouping of BSRC patients, removal of heterophilic immunoglobulins, and use of microarray assays. In addition, n size is small, but the study is still undergoing additional recruitment. IL-23 is involved in chronic inflammation and the pathogenesis of autoimmune disease, probably via its maintenance and development of pathogenicity of Th17 cells, and promotion IL-17 and IL-6 production. Our finding suggests that elevations in peripheral levels of IL-23 in BSRC patients may play a role in promoting locally elevated levels of IL-17 and pro-inflammatory cytokines in the eye. If this is true, systemic therapy targeted against IL-23 may prove to be useful in the management of BSRC.

CONCLUSION

Serum levels of IL-23 are significantly elevated in active BSRC patients naïve to IMT. Taken together with other studies, these results suggest that the IL-23/IL-17 pathway may play an important role in the pathophysiology of BSRC.

BIBLIOGRAPHY

Efficacy of Combined Cyclosporine A and Mycophenolate Mofetil in the Treatment of Patients with Birdshot Retinochoroidopathy

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INTRODUCTION

BSRC is a bilateral, chronic idiopathic posterior uveitis characterized by vitritis and multiple hypopigmented lesions (Figure 1). Patients usually complain of progressive, debilitating vision loss even in the face of 20/20 Snellen visual acuity, which reflects the progression of retinal damage; moreover, this may occur in the absence of evident clinical findings (i.e. frank vitritis or vasculitis). Untreated patients eventually progress to functional blindness. Serial ERG and fluorescein angiograms are of utmost help to assess the progression of deterioration of retinal function and to support clinical decision making in treating patients with BSRC.

METHODS

• Retrospective, non-comparative, interventional case-series.
• Eighty eyes of 40 patients with BSRC who received CsA and MM for a minimum of one year.
• All patients were followed for at least five visits during the study.
• Outcome measures included BCVA logMAR, vitreous inflammation, FA features, ERG recordings, reported side effects to therapy, and number of relapses.
• Student’s t-test, Pearson’s chi-square test, and Fisher’s exact test were used for statistical analysis.

RESULTS

Several IMT regimens have been explored in efforts aimed at induction of a durable remission without the risks of long-term corticosteroid therapy. However, there is no published consensus providing a guideline for BSRC treatment. We have reported the efficacy of CsA monotherapy at induction of remission; however, this required years of treatment rather than durable remission or cure after 2 years of therapy. We herein report the results of the use of combination of CsA and Mycophenolate mofetil in 40 patients with BSRC during a period of 12-months.

CONCLUSIONS

Several attempts to manage BSRC using CsA alone, MTX alone or other different IMTs have been reported. In the current series, adding MM to CSA resulted in inflammatory control and ERG parameters stabilization in 92.5% of patients in less than one year. All of these patients were able to maintain remission off any kind of steroids after one year. The long term results suggest that a 2-year period on combined therapy followed by a slow taper of IMT induces durable remission in a majority of patients (measured by FA/ERG). Moreover, this strategy has allowed 6 of our patients to be off IMT for a median of over 3 years. Frequent follow up and high risk blood monitoring are mandatory for detecting occult side effects and reducing the likelihood of needing to discontinue therapy.

We analyzed the results of combined therapy over a total follow up time of 52.6 months (19.7–60.0), 65% of patients did not relapse over the entire follow-up time. 27% of these patients achieved durable remission off IMT after at least 2 years of treatment (median f/u time off IMT was 36.67 months). Moreover, another 29.7% of patients continue to be in remission while on long term IMT. Nine patients had to be transitioned to biologic response modifiers due to severe fatigue. Other side effects were transient and resolved after lowering or withholding IMT for a few weeks in all patients. Frequent follow up and high risk blood monitoring are mandatory for detecting occult side effects and reducing the likelihood of needing to discontinue therapy. These data suggest that CsA/MM therapy is well-tolerated and efficacious in controlling inflammation and stabilizing vision for the majority of patients with BSRC.
Chronicity of BSRC, systemic corticosteroid therapy is not characterized by multiple, distinctive, ovoid, hypopigmented lesions. Mean duration on Infliximab was 13.55 months. Prior to starting infliximab, the mean duration of disease was 58.62 months. Prior to infliximab therapy, all patients received and failed conventional immunosuppressive therapy. Ten patients had received another biologic agent.

Methods

All refractory birdshot retinochoroidopathy patients from July 2005 to October 2011 were identified from retrospective chart review. All patients received 4-5 mg/kg of infliximab at 4-8 week intervals. Demographic data, use of immunosuppressive drugs, biologic agents, and reason for conventional therapy discontinuation were gathered. Disease activity monitoring included signs of ocular inflammation, fluorescein angiography (FA) evidence of retinal vasculitis or papillitis, indocyanine green angiography (ICG) evidence of active choroidal neovascularization, electroretinography (ERG) parameters indicative of active or worsening of retinal functions, and optical coherence tomography (OCT) findings indicative of static or worsening macular edema were recorded. The outcome features of primary interest were absence of all evidence of active inflammation, improved visual acuity (VA), and presence of cystoid macular edema (CME). Corticosteroid-sparing success was defined as inactive inflammation after tapering prednisone to ≤ 10 mg per day. We assessed the outcomes of infliximab therapy at 6 months and 1 year follow up. Adverse events of infliximab were also tabulated.

Results

Mean duration of disease before starting infliximab was 58.62 months. Mean duration on infliximab was 13.55 months. Prior to infliximab therapy, all patients received and failed conventional immunosuppressive therapy. Ten patients had received another biologic agent.

Discussion

The effectiveness of infliximab in BSRC has been reported in few small case series so far. Suhler and colleagues found infliximab to be effective in refractory uveitis with 78% success rate at 10 weeks and 77% at 2 years. Baughman et al. reported that 14 patients with various ocular inflammatory conditions had improvement of inflammation, visual acuity, and steroid-sparing success. The data suggest that Infliximab may be effective for controlling inflammation in otherwise treatment refractory cases of BSRC.

After initiating Infliximab, control of inflammation was achieved in 81.82% at 6 months and 75% at 1 year follow up. Three patients had active inflammation during therapy. Cystoid macular edema decreased from 22.73% at baseline to 13.89% at 6 months and 5.56% at 1 year after receiving the drug. Initial visual acuity 20/40 or better was found in 34 eyes (84.09%). At 6 months and 1 year, 91.67% and 94.44% of eyes respectively had VA 20/40 or better.
INTRODUCTION

Birdshot retinochoroidopathy (BSRC) is a chronic, bilateral, posterior form of uveitis which is a relatively rare and occurs in 7.9% of patients with posterior uveitis. It tends to occur between 35 - 70 years of age, with the average age of presentation being 50 years. [1] Management of the patient with BSRC can be challenging as the disease has a chronic protracted course, which makes it difficult to assess progression, effect of treatment and need for further treatment. Disease activity is monitored in one or more of the following ways: slit-lamp examination, fluorescein angiography (FA) fig 1, indocyanine green angiography (ICG) and electroretinograms (ERG) fig 2. The purpose of this study was to evaluate the risk factors for relapse of BSRC patient after treatment with immunomodulatory therapy.

METHODS

Chart review of 37 patients diagnosed with BSRC who were treated successfully with immunomodulatory therapy (IMT) and those who remained in remission for one year off all IMT was performed. The following data were noted for each patient: age, gender, presence of HLA-A29 haplotype, type of IMT, visual acuity (VA), any associated systemic disease and family history of other autoimmune diseases. We subdivided the patients into two groups. Group “A” included patients who continued to be in remission off immunomodulatory therapy for more than 1 year. Group “B” were patients who had relapse of disease after a durable drug free remission of at least 1 year. There is a statistically significant difference in age at disease onset between the two groups; majority of the patients in Group A were above 50 years of age (n=17) compared to Group B (n=2), (P<0.02). Greater number of patients in Group A had symmetric eye involvement (n=22) as compared to Group B (n=4) (P=0.05).

Table. Analysis of risk factors in the Non recurrent and the recurrent group

<table>
<thead>
<tr>
<th>Gender</th>
<th>Groups A N=25</th>
<th>Groups B N=10</th>
<th>P value (Chi square)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>12</td>
<td>7</td>
<td>0.167(1.908)</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Age ≥ 50</td>
<td>17</td>
<td>2</td>
<td>0.02*(5.392)</td>
</tr>
<tr>
<td>Age &lt; 50</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>HLA-A29 positive test</td>
<td>4</td>
<td>0</td>
<td>0.137(2.211)</td>
</tr>
<tr>
<td>Symmetrical</td>
<td>22</td>
<td>4</td>
<td>0.85*(7.776)</td>
</tr>
<tr>
<td>Asymmetrical</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Right eye</td>
<td>8</td>
<td>1</td>
<td>0.573</td>
</tr>
<tr>
<td>Left eye</td>
<td>9</td>
<td>3</td>
<td>0.269</td>
</tr>
<tr>
<td>Family H/o other autoimmune disease</td>
<td>13</td>
<td>7</td>
<td>0.236(1.403)</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Type of IMT</td>
<td>21</td>
<td>9</td>
<td>0.353(0.892)</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>24</td>
<td>8</td>
<td>0.081(16.719)</td>
</tr>
<tr>
<td>Ciclosporin</td>
<td>25</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Duration of IMT mean(SD)</td>
<td>66.7±(49.28)</td>
<td>25.86±(14.06)</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

In eighteen cases, immunosuppressive drugs were used alone (without corticosteroids), 16 of these cases were in Group A and only 2 were in Group B. This was statistically significant (P=0.002). The mean duration of IMT was significantly longer in Group A compared to Group B (60.7 ±49.28 vs. 25.86 ±14.06; P 0.002).

RESULTS

There were 19 males (51.4%) and 18 female (48.6%). The mean age was 54 (SD 11.019) and 47.00 (SD 11.225) in Group-A and Group-B, respectively, with a range of 25 to 78 years.

There is a statistically significant difference in age at disease onset between the two groups; majority of the patients in Group A were above 50 years of age (n=17) compared to Group B (n=2), (P<0.02). Greater number of patients in Group A had symmetric eye involvement (n=22) as compared to Group B (n=4) (P=0.05). The two groups did not differ in terms of family history of autoimmune diseases and positive history of other autoimmune and systemic diseases.

In eighteen cases, immunosuppressive drugs were used alone (without corticosteroids), 16 of these cases were in Group A and only 2 were in Group B. This was statistically significant (P=0.002). The mean duration of IMT was significantly longer in Group A compared to Group B (60.7 ±49.28 vs. 25.86 ±14.06; P 0.002).

DISCUSSION

Our study demonstrates that patients who relapsed after successful treatment with immunomodulatory drugs and a durable remission of 1 year off IMT had early age of disease onset<50 years, asymmetric visual acuity at presentation, and received immunomodulatory treatment for lesser duration as compared to patients who continued to be in remission. Patarroyo et al. have reported that there are certain autoimmune diseases where early onset of disease is associated with poor prognosis. [2] Similarly we found that patients with early onset disease<50 years had comparatively greater chances of relapse. Rothova and associates have reported strong association between VA at disease onset and the visual outcome after 5-10 years in BSCR patients. Symmetric visual acuity at disease onset was found in 75.4% patients. [3] Patients with asymmetric visual acuity at presentation had greater chances of relapse. Another interesting finding of our study was that (60%) patients in the relapse group received oral corticosteroids as compared to (7.4 %) in remission group (P=0.002). This finding is similar to the optic neuritis treatment trial (ONTT) which showed increased recurrence in patients of optic neuritis treated with oral corticosteroids. [4]

CONCLUSION

To conclude our study suggests that the patients with early age of onset of BSRC disease<50 years, those treated with oral corticosteroids and shorter duration of IMT are more likely to relapse over time.

BIBLIOGRAPHY

Regulatory T-Cells In Peripheral Blood of Patients With Birdshot Retinochoroidopathy

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INTRODUCTION

Birdshot retinochoroidopathy (BSRC) is a chronic inflammatory disease which appears to be isolated to the eyes, causing distinct bilateral chorioretinal lesions, and progressive vision loss, treatable with systemic immunomodulatory therapy (IMT). The pathogenesis has not been elucidated, but lymphocytic infiltration has been found on BSRC histopathology1, and the role of retinal autoreactivity has been suggested2. BSRC has been strongly linked to human leukocyte antigen A29 (HLA-A29). At present, the most probable hypothesis is that HLA-A29, by molecular mimicry, predisposes to autoimmunity against retinal and/or choroidal antigens, with an infectious or non-infectious agent acting as a trigger to the immune reaction. As yet, the trigger of presumed autoreactivity in BSRC is not known. Regulatory T cells (Tregs) are another important element described in the development of autoimmunity and CD4+ CD25+ FOXP3+ T cells represent this Treg comportment. We assessed the percentage of T regulatory cells in the peripheral blood of patients with active BSRC and compared this to normal persons.

METHODS

This was a single center prospective study of 5 BSRC patients, and 5 controls, approved by the New England Institutional Review Board. Blood from patients between the ages of 30 and 70 years, with the diagnosis of birdshot retinochoroidopathy, naïve to IMT, and healthy individuals as controls was collected from April 2010 to December 2011. The diagnosis of BSRC was based on the research criteria of an international consensus conference2, and HLA-A29 positivity. All the patients were enrolled from Dr. C. Stephen Foster’s patient pool at the Massachusetts Eye Research & Surgery Institution. Flow cytometry was used for analysis of the percentage of CD4+ CD25+ FOXP3+ T regulatory cells in blood specimens. A sequential gating method was used to analyze FOXP3 data. First, a gate was placed around the population of events that are CD45+ and low in side scatter and were labeled as lymphocytes. Cells that fell within the lymphocyte gate and were CD3+CD4+ were labeled CD4+ T cells. CD25 expression on the CD4+ T cell population was gated as follows: CD25high+, CD25low+ and CD25-. FOXP3 expression on all three populations was analyzed and the percentage of FOXP3 positive versus negative was determined. Two-tailed T test with a P value of 0.05 for significance was used.

RESULTS

Blood CD4+ CD25+ FOXP3+ T regulatory cells were detectable in all active BSRC patients naïve to IMT as well as controls. The percentage of Tregs in BSRC patients was lower (86.2%) as compared to controls (100%). These results were statistically significant with a p value of less than 0.008.

DISCUSSION

The role of CD4+ CD25+ FOXP3+ T regulatory cells has been established in the pathogenesis of systemic autoimmune disorders such as diabetes mellitus, rheumatoid arthritis and systemic lupus erythematosus. Ocularly, Tregs were implicated in diseases such as Behcet’s4 and Vogt-Koyanagi-Harada (VKH) syndrome5. Significantly lower percentages of CD4+ CD25+ FOXP3+ T regulatory cells were found in patients with active VKH and Behcet’s disease. Similarly, we have demonstrated for the first time in a prospective study, the percentages of CD4+ CD25+ FOXP3+ T regulatory cells in patients with birdshot retinochoriodopathy and have established that the percentage is lower in BSRC patients. This may suggest that CD4+ CD25+ FOXP3+ T regulatory cell dysregulation might play a role in BSRC. Further studies are needed. The number of patients in our study was low.

CONCLUSION

Blood levels of CD4+ CD25+ FOXP3+ T regulatory cells are significantly lower in active BSRC patients naïve to IMT as compared to healthy individuals. Thus, we conclude that Treg deficiency may play an important role in the pathophysiology of BSRC.

BIBLIOGRAPHY