Elevated CD1c+ Myeloid Dendritic Cell Proportions Associate With Clinical Activity and Predict Disease Reactivation in Noninfectious Uveitis

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PURPOSE. To test the association between elevated proportions of CD1c+ myeloid dendritic cells (mDCs) and disease activation/reactivation in noninfectious uveitis.

METHODS. Noninfectious uveitis patients ($n = 89$) and healthy controls ($n = 111$) were recruited. The proportion of CD1c+ mDCs in the total dendritic cell (DC) population of peripheral blood was measured by flow cytometry (CD1c+ mDCs gated on Lineage 1+HLADR+ DCs). Disease activity was assessed per Standardization of Uveitis Nomenclature criteria. Uveitis reactivation was ascribed to clinically quiescent patients who developed reactivation of intraocular inflammation within 6 months.

RESULTS. The proportions of CD1c+ mDCs were increased in noninfectious uveitis patients, especially in active disease, compared to healthy controls. This CD1c+ mDC elevation was not associated with underlying systemic diseases, anatomic locations of uveitis, medications, or demographic factors. Longitudinal data showed that the dynamics of CD1c+ mDC levels were correlated with disease activity. The average proportion of CD1c+ mDCs in active uveitis patients was 60% so we set this as the cutoff between high and low CD1c+ mDC levels. Although 74% of quiescent patients had low proportions of CD1c+ mDCs, 26% still had high proportions. Quiescent patients with high CD1c+ mDC proportions showed increased risk of disease reactivation, compared to quiescent patients with low CD1c+ mDC proportions.

CONCLUSIONS. Increased proportions of CD1c+ mDCs were associated with clinical activity, and quiescent patients with elevated CD1c+ mDCs were more likely to undergo reactivation. This suggests that CD1c+ mDC proportion may be a potential biomarker for assessing clinical activation and reactivation in noninfectious uveitis.

Keywords: CD1c+ myeloid dendritic cells, noninfectious uveitis, clinical activity, disease reactivation

Noninfectious uveitis (uveitis) consists of a group of inflammatory eye diseases that often require local and/or systemic immunosuppression to adequately control intraocular inflammation and prevent blindness.[1] While the pathophysiology is not completely understood, experimental models of autoimmune uveitis suggest that CD4+ T-cell activation by uveitis-specific antigens plays a major role.[2] Therefore, both topical and systemic immunosuppressive treatments have been used extensively to control uveitis in patients. In the short term, glucocorticoids are the mainstay of treatment and may be required at high doses to adequately treat the disease.[3] While very effective, high-dose glucocorticoids are used sparingly owing to their significant systemic adverse effects, and they must be carefully tapered. Timing and rapidity of the taper is based primarily on symptoms and examination findings. Reduction or discontinuation of antimetabolite or biologic immunosuppressive medications also helps uveitis patients with quiescent disease avoid potentially life-threatening side effects. Unfortunately, reactivation of uveitis is common during or after tapering immunosuppressive medications. Currently, there is no way to precisely predict the likelihood of clinical reactivation.[4–6] Therefore, there is a need to develop reliable methods to predict reactivation of uveitis.

Dendritic cells (DCs) present antigens to T cells to induce adaptive immune responses.[1,7] Different types of DCs play different roles in autoimmune disease. CD1c+ myeloid dendritic cells (mDCs) are one of the major DC subtypes in humans.[1,8] We have previously observed that proportions of CD1c+ mDCs are increased in uveitis patients compared to healthy controls (HCs) in a small cohort.[9] Furthermore, a high level of CD1c expression on mDCs is strongly associated with increased HLADR expression and decreased mDC antigen uptake, suggesting that CD1c+ mDCs are activated and mature in uveitis patients.[1]

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TABLE. Characteristics of Uveitis Patients and Healthy Controls

Parameters	Uveitis (n = 89)	HCs (n = 111)	P Value
Age, average (range), y	45 (12-78)	46 (20-87)	0.50
Sex, n (%)			0.0005
Male	35 (39)	71 (64)	
Female	54 (61)	40 (36)	
Race, n (%)			0.94
Caucasian	36 (40)	66 (59)	
African American	37 (42)	36 (32)	
Others	16 (18)	9 (8)	
Anatomic type of uveitis, n (%)		N/A	
Anterior	5 (6)		
Intermediate	9 (10)		
Posterior	36 (40)		
Panuveitis	39 (44)		
Disease association, n (%)		N/A	
Sarcoidosis	21 (24)		
Idiopathic	25 (28)		
Birdshot	20 (22)		
VKH	9 (10)		
Behcet's disease	5 (6)		
Others	9 (10)		
Systemic therapy, n (%)		N/A	
No therapy	24 (27)		
Glucocorticoids only	11 (12)		
Other immunosuppressive therapy	30 (34)		
Glucocorticoids + other immunosuppressive therapy	24 (27)		
Active uveitis, n (%)	26 (29)	N/A	

N/A, not applicable.

In this study, we investigated whether CD1c$^+$ mDC proportion is associated with clinical activity, and perhaps more importantly, whether CD1c$^+$ mDC proportion can predict disease reactivation in clinically quiescent patients.

MATERIALS AND METHODS

Study Design

Noninfectious uveitis patients and healthy controls were recruited from the National Eye Institute (NEI) clinic and the National Institutes of Health (NIH) Clinical Center Blood Bank, respectively. Patients with infectious uveitis or systemic disease lacking ocular involvement were excluded from this study. Healthy controls were required to be nonpregnant (if female) with no history of heart, lung, kidney, or hematologic diseases and no history of intravenous injection drug use, high-risk activity, or experimental drug use in order to donate blood to the Blood Bank. All study subjects provided written informed consent before participation in the study commenced. The study adhered to the tenets set forth in the Declaration of Helsinki. All protocols were approved by the NIH Institutional Review Board.

Clinical Evaluation

Disease activity was assessed by the treating physicians and reported according to the "Standardization of Uveitis Nomenclature" (SUN criteria).[10] For the purpose of this study we categorized activity as follows: quiescent (0 anterior chamber cell and 0 vitreous haze) and active (0.5+ or greater anterior chamber cells and/or any vitreous haze). Those patients that reactivated within 6 months after the initial evaluation were retrospectively categorized as clinical reactivation.

CD1c$^+$ mDC Staining and Flow Cytometry

Peripheral blood (0.4 mL) was obtained from each participant for flow cytometry analysis. CD1c$^+$ mDCs and plasmacytoid dendritic cells (pDCs) were identified as the Lineage Cocktail 1 (Lin1)$^-$HLADR$^+$CD1c$^+$ and Lin1$^-$HLADR$^+$CD303$^+$ populations, respectively, as previously reported.[1,8,9] Antibodies were purchased from Miltenyi Biotec (San Diego, CA, USA) or BD Biosciences (San Diego, CA, USA). The same procedure for handling blood samples was used for HCs and patients.

Mixed Lymphocyte Reaction (MLR) Assay

In the MLR assay, monocyte-derived dendritic cells (MoDCs) with or without p38-MAPK inhibitor (SB203580; Cell Signaling, Danvers, MA, USA) treatment were cocultured with allogeneic CD4$^+$ or CD8$^+$ T cells for 5 days with concurrent stimulation of 1 μg/mL anti-CD3 (clone UCHT1; BD Biosciences). T-cell proliferation was characterized by flow cytometry as the percentage of cells exhibiting carboxyfluorescein succinimidyl ester (CFSE) fluorescence dilution.

Statistical Analysis

The data are presented as mean ± standard deviation (SD). Statistical comparisons were performed by using the Student's *t*-test when analyzing data for two groups or analysis of variance (ANOVA) when analyzing data for three or more groups. Receiver operating characteristic (ROC) curves were plotted to evaluate the accuracy of CD1c$^+$ mDCs proportion as a risk classifier for clinical activity and predicting future activity. The measurements with the largest area under the curve (AUC) were chosen as a significant classifier and, therefore, eligible for calculation of sensitivity and specificity (χ^2 test). $P < 0.05$ was considered to be significant. Analyses were accomplished by using Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Study Participant Characteristics

Noninfectious uveitis patients ($n = 89$) seen at the NEI and healthy controls ($n = 111$) from the NIH Blood Bank between January 2012 and July 2015 were included in the study. Demographics (age, sex, and race) and clinical information (underlying systemic diseases, anatomic locations of uveitis, medications, and disease activity) of patients were recorded (Table). CD1c$^+$ mDC levels were collected longitudinally on 44 of 89 patients who visited the eye clinic multiple times throughout the study period. The frequency of visits depended on their disease activity, disease development, and response to therapy. In general, patients with active disease were followed up every 2 weeks and quiescent patients were followed up every 2 months. Therefore, some of patients were able to give blood samples at least twice.

CD1c$^+$ mDC Proportions Were Elevated in Noninfectious Uveitis Patients

Consistent with our prior observation, the proportions of CD1c$^+$ mDCs in the total DC populations were significantly
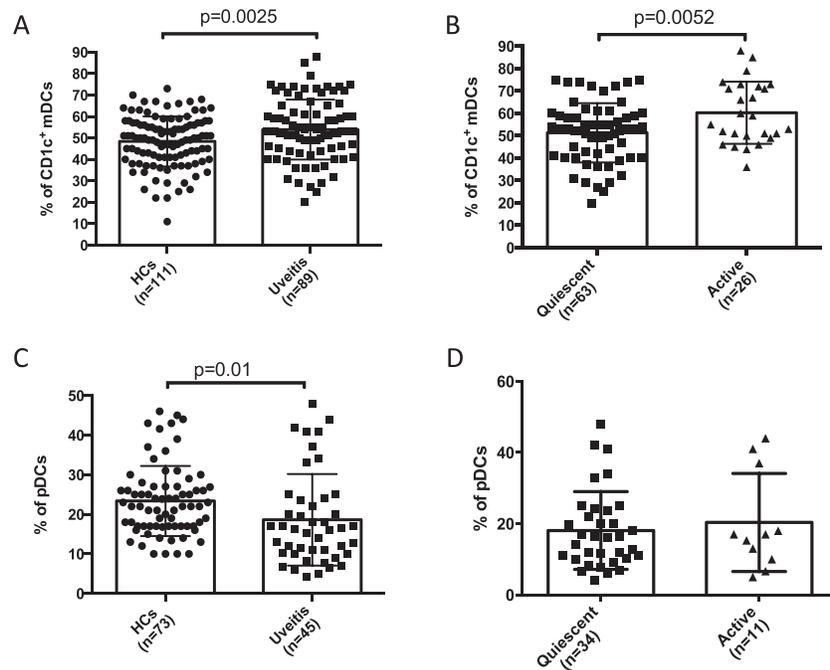
FIGURE 1. Proportions of CD1c$^+$ mDCs and pDCs were measured in noninfectious uveitis patients. Lin1$^-$HLADR$^+$ total DCs were identified by flow cytometry. CD1c$^+$ mDCs and CD303$^+$ pDCs were gated on total blood DC population (Lin1$^-$HLADR$^+$). (**A**) The proportions of CD1c$^+$ mDCs were compared between HCs and noninfectious uveitis patients (uveitis). (**B**) The percentages of CD1c$^+$ mDCs were compared between quiescent and active uveitis patients. (**C**) The proportions of CD303$^+$ pDCs were compared between HCs and uveitis patients. (**D**) The proportions of CD303$^+$ pDCs were compared between quiescent and active uveitis patients. The data are presented as mean ± SD. Unpaired Student's *t*-test was used for statistical analysis.

elevated in uveitis patients when compared to HCs (HCs = 111, uveitis = 89, *P* = 0.0025; Fig. 1A). Furthermore, CD1c$^+$ mDCs were significantly higher in patients with active disease as compared to those who were clinically quiescent (*P* = 0.0052; Fig. 1B). In addition to mDCs, pDCs also play a role in uveitis although their function is not fully understood yet.[8] To assess any relative changes in the pDC populations in uveitis patients compared to HCs, we also measured the proportions of pDCs in the total DC populations by flow cytometry. In contrast to CD1c$^+$ mDC proportions, pDCs proportions were decreased in uveitis patients compared to those in HCs (*P* = 0.01; Fig. 1C), and no difference was detected between patients with active and quiescent uveitis (Fig. 1D). In summary, CD1c$^+$ mDC proportions were elevated in uveitis patients. Additionally, CD1c$^+$ mDCs proportions were associated with disease activity in uveitis patients.

Elevated CD1c$^+$ mDC Proportions Were Independent of Uveitis Classifications and Systemic Immunosuppression

A variety of underlying systemic disorders are associated with noninfectious uveitis. Indeed, the diversity in underlying systemic disease implies that there are key differences in pathogenesis among these diseases, but the commonality of noninfectious ocular inflammation also implies common mechanisms. Subgroup univariate analyses revealed no difference in CD1c$^+$ mDC proportions among uveitis patients with different underlying diseases including sarcoidosis, idiopathic, Birdshot, Vogt-Koyanagi-Harada (VKH), Behcet's diseases, or others (Fig. 2A), and different anatomic locations (anterior, intermediate, posterior, or panuveitis; Fig. 2B).

During the study period, 73% of patients were on immunosuppressive therapy, which is known to alter T-cell phenotype and function.[11,12] Therefore, we also investigated the effects of treatment with immunosuppressive medications on CD1c$^+$ mDC proportions and found no significant difference (Fig. 2C). There was also no difference on CD1c$^+$ mDC proportions between patients treated with different doses of prednisone ranging from no prednisone, ≤10 mg prednisone per day, 11 to 20 mg per day, and >20 mg per day (Fig. 2D). Moreover, in vitro treatment of MoDCs with dexamethasone or cyclosporine A did not alter CD1c$^+$ mDC proportions or mean fluorescence intensity (Figs. 2E, 2F). In summary, CD1c$^+$ mDC elevation was not significantly associated with the etiologic and anatomic classifications of uveitis, or types and dosages of immunosuppressive therapy.

Elevated CD1c$^+$ mDC Proportions Were Not Associated With Age, Sex, or Race

Previous publications have shown that sex differences may alter susceptibility to autoimmune disease[13] and that aging is associated with immunosenescence.[14] Therefore, subgroup univariate analyses of CD1c$^+$ mDC proportions in HCs (Figs. 3A–C) and uveitis patients (Figs. 3D–F) were performed by age (Figs. 3A, 3D), sex (Figs. 3B, 3E), and race (Figs. 3C, 3F). CD1c$^+$ mDC proportions were not associated with these demographic factors.

Dynamic Proportions of CD1c$^+$ mDCs Were Associated With Clinical Activity in Longitudinal Data

Among 44 patients who had longitudinal data of CD1c$^+$ mDC levels with multiple time points, CD1c$^+$ mDC proportions were not altered significantly in those patients who were initially clinically quiescent (Q) and remained quiescent (Q-Q,
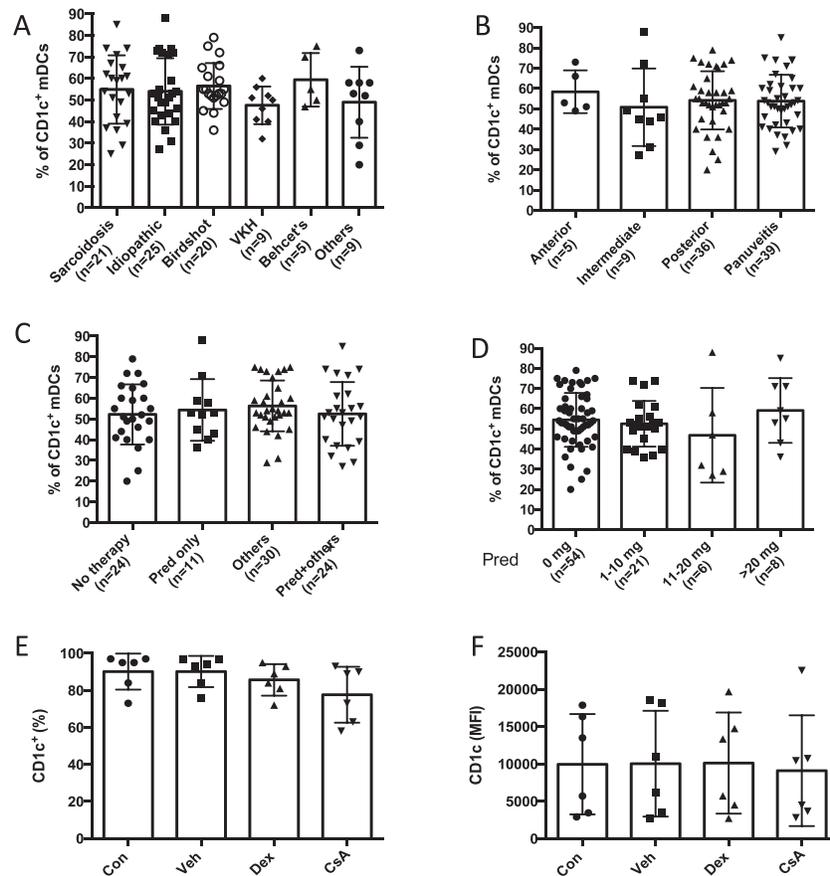
FIGURE 2. Proportions of CD1c⁺ mDCs were independent of uveitis classifications and medications. Uveitis patients were divided into subgroups on the basis of their underlying systemic diseases and locations of uveitis. (**A**) Proportions of CD1c⁺ mDCs were compared in different systemic diseases including sarcoidosis, idiopathic, Birdshot, VKH, Behcet's disease, and others (ANOVA test, $P = 0.40$). (**B**) Proportions of CD1c⁺ mDCs were compared in anatomic locations of uveitis including anterior, intermediate, posterior, and panuveitis (ANOVA test, $P = 0.76$). (**C**) CD1c⁺ mDC proportions were compared among four subgroups: no therapy, prednisone only (Pred only), other systemic immunosuppressive treatment (others), and prednisone plus other systemic immunosuppressive treatment (Pred + others) (ANOVA test, $P = 0.75$). (**D**) CD1c⁺ mDC proportions were compared in the subgroups on the basis of the dosage of prednisone used daily including 0 mg, 1 to 10 mg, 11 to 20 mg, and >20 mg (ANOVA test, $P = 0.16$). (**E, F**) Monocyte-derived dendritic cells were treated with dexamethasone (Dex, $n = 6$), cyclosporine (CsA, $n = 6$) for 24 hours. Monocyte-derived dendritic cells without treatment were the control group (Con, $n = 6$) and those treated with dimethyl sulfoxide (DMSO) were the vehicle group (Veh, $n = 6$) because Dex and CsA were dissolved in DMSO. Proportions of CD1c⁺ mDCs and mean fluorescence intensity (MFI) of CD1c on mDCs were calculated by flow cytometry. The data are presented as mean ± SD (ANOVA test, $P = 0.23$ [**E**]; $P = 0.99$ [**F**]).

$n = 25$, $P = 0.35$; Fig. 4A), or in patients with active disease (A) who remained active between blood draws (A-A, $n = 10$, $P = 0.67$; Fig. 4B). However, active patients who became quiescent after treatment showed decreased CD1c⁺ mDC proportions (A-Q, $n = 14$, $P = 0.03$; Fig. 4C) and quiescent patients showed increased CD1c⁺ mDC levels when they developed active uveitis after tapering off medications (Q-A, $n = 4$, $P = 0.02$; Fig. 4D). Plotting the average level of CD1c⁺ mDC proportions in continually quiescent patients (Q-Q), continually active patients (A-A), and patients with changes in disease activity from active to quiescent (A-Q) or quiescent to active (Q-A), showed continually low, continually high, significantly decreasing, and significantly increasing CD1c⁺ mDC proportions, respectively (Fig. 4E). Furthermore, fold changes of CD1c⁺ mDC proportions between two time points from each patient were calculated in the groups of Q-Q (Q/Q = 1.05), A-A (A/A = 1.07), A-Q (Q/A = 0.88) and Q-A (A/Q = 1.40). The fold changes in the Q-A group were significantly increased as compared to groups of Q-Q and A-A (Q-A versus Q-Q, $P = 0.017$; Q-A versus A-A, $P = 0.022$; Fig. 4F). The fold changes in A-Q group were decreased as compared to the groups of Q-Q and A-A although not statistically significantly (Fig. 4F). These results suggest that

dynamic levels of CD1c⁺ mDC proportions are associated with disease activity.

To evaluate the potential role of CD1c⁺ mDC proportion as a biomarker for uveitic activity, an ROC curve was plotted, resulting in an AUC of 0.64 ($P = 0.035$; Fig. 4F), sensitivity of 40%, and specificity of 77%. Further investigation is needed to evaluate whether CD1c⁺ mDC proportion could be a biomarker for uveitic activity.

Elevated CD1c⁺ mDC Proportions Were Associated With Disease Reactivation

Although current uveitic activity can be determined by clinical examination and imaging findings, an oncoming activity is difficult to predict. There is currently no adequate laboratory or clinical biomarker to predict reactivation. In our cross-sectional data (Fig. 1B), we noticed that while most quiescent patients had low proportions of CD1c⁺ mDCs, 26% of quiescent patients still had CD1c⁺ mDC proportions that were higher than the average proportion in active uveitis patients (60% of CD1c⁺ mDCs). We hypothesized that these quiescent patients with elevated CD1c⁺ mDC proportions were at
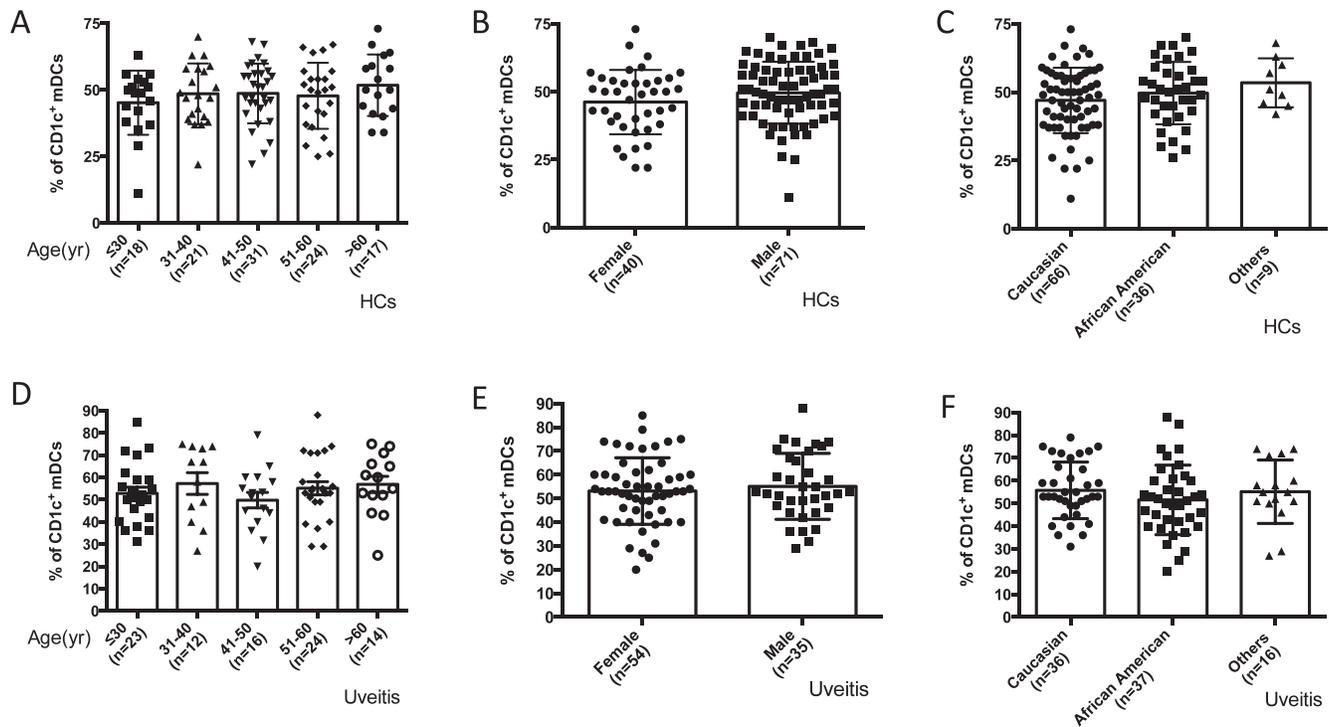
FIGURE 3. CD1c+ mDC proportions were independent of age, sex, or race. CD1c+ mDC proportions from HCs (**A–C**) or uveitis patients (**D–F**) were divided into subgroups according to their ages including ≤30, 31 to 40, 41 to 50, 51 to 60, and >60 years (**A**, **D**); sex including female and male (**B**, **E**); and race including Caucasian, African American, and others (**E**, **F**). The data are presented as mean ± SD. Analysis of variance test was used for statistical analysis in (**A**), (**C**), (**D**), and (**F**). Unpaired Student's *t*-test was used in (**B**) and (**E**).

increased risks for disease reactivation. To test this hypothesis, we divided quiescent patients ($n = 34$) into high ($n = 10$) and low ($n = 24$) CD1c+ mDC proportion subgroups on the basis of a cutoff of CD1c+ mDC proportion in total DC population, which was 60%. This cutoff was the average proportion of CD1c+ mDCs in active uveitis patients. Interestingly, 6 of 24 quiescent patients (25%) had disease reactivation in the low CD1c subgroup compared to 7 of 10 patients (70%) in high CD1c subgroup ($P = 0.01$; Fig. 5A). Additionally, the proportions of CD1c+ mDCs among patients who eventually reactivated were higher than for those who remained clinically quiet ($P = 0.02$; Fig. 5B), indicating that CD1c+ mDC proportions were associated with subsequent uveitis reactivation. Given the potential clinical use of CD1c+ mDC proportion to detect clinical reactivation, a ROC curve was plotted, with an AUC of 0.68 ($P = 0.03$; Fig. 5C), sensitivity of 44%, and specificity of 90%.

Monocyte-Derived Dendritic Cells With Low CD1c+ Proportions Associated With Impaired Allogeneic T-Cell Proliferation

To investigate whether the elevated CD1c+ mDC proportions observed in noninfectious uveitis patients had effects on adaptive immune response, we performed an in vitro study to evaluate the effects of CD1c expression on mDC–T-cell interactions. Our published data have shown that blocking p38-MAPK pathway during MoDC differentiation impairs CD1c expression on MoDCs in vitro.[1] In this experiment, proportions of CD1c+ MoDCs were decreased after p38-MAPK inhibitor treatment (Fig. 6A), and then these MoDCs (p38i-MoDCs) cocultured with allogeneic CD4+ or CD8+ T cells. Monocyte-derived dendritic cells without p38 MAPK inhibitor treatment were used as controls. T-cell proliferation was

significantly reduced in cultures with MoDCs treated with p38-MAPK inhibitor (Figs. 6B, 6C).

DISCUSSION

Uveitis is a significant cause of ocular morbidity and vision loss, and the lack of adequate biomarkers for predicting disease recurrence makes long-term clinical management challenging. Our study showed that CD1c+ mDC proportions were increased in uveitis patients and that this elevation appeared to be correlated with clinical activity. In univariate analyses, high proportions of CD1c+ mDCs were significantly associated with disease activity, while other possible clinical parameters, such as underlying systemic diseases, anatomic locations of uveitis, current immunosuppressive medications, age, sex, and race, were not. Surprisingly, 26% of quiescent patients retained a high proportion of CD1c+ mDCs. These patients subsequently had a higher rate of disease reactivation as compared to quiescent patients with low CD1c+ mDC proportions. Thus, CD1c+ mDC proportion may have the potential to be a biomarker for uveitic disease activity and reactivation.

Increased CD1c+ mDC number and activity have been demonstrated in a variety of autoimmune and other immune response–related diseases such as inflammatory bowel disease, rheumatoid arthritis, and asthma.[15-17] In our previous publications, we have reported elevated proportions of CD1c+ mDCs in a small cohort of noninfectious uveitis patients when compared to healthy controls[1] and have demonstrated that CD1c proportion rises and decreases with changes in clinical uveitis activity.[9] In this study, with a larger sample size, we supported our previous findings of (1) elevated CD1c+ mDC proportions in uveitis patients compared to healthy controls and (2) a correlation between CD1c+ mDC proportion and disease activity. Owing to the existing evidence that aging
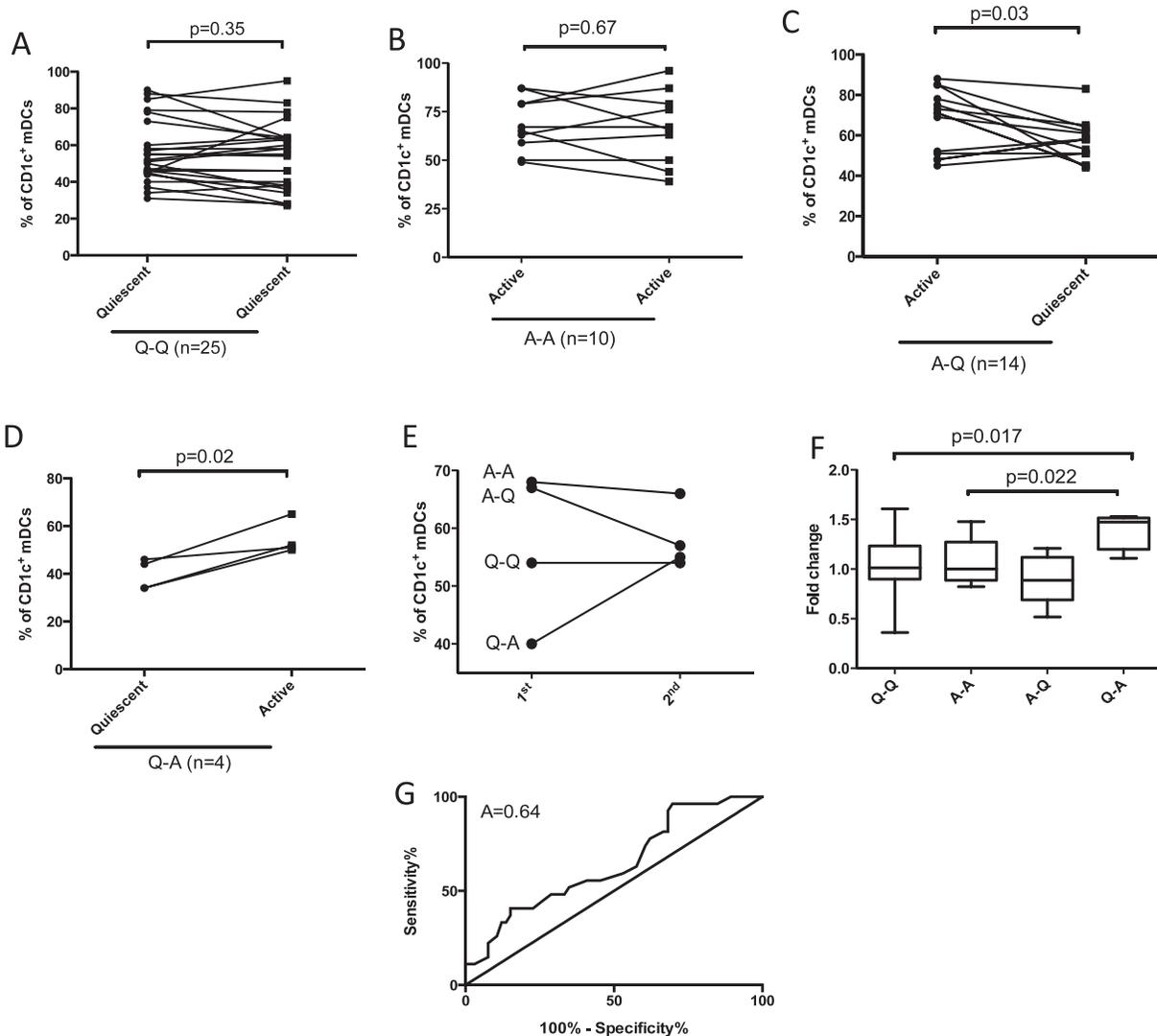
FIGURE 4. Dynamic proportions of CD1c$^+$ mDCs were observed in uveitis patients with different disease status. Blood samples were collected at multiple time points from each patient longitudinally, followed by the measurement of CD1c$^+$ mDC proportions by flow cytometry. The data were compared in the groups of patients who maintained the quiescent uveitis ([**A**], quiescent to quiescent, Q-Q), of patients whose diseases were active during sampling ([**B**], active to active, A-A), of patients whose disease activity changed from the active to quiescent ([**C**], A-Q) or from the quiescent to active ([**D**], Q-A). (**E**) The average proportion of CD1c$^+$ mDCs from each group (Q-Q, A-A, A-Q, Q-A) was compared. *Lines Q-Q, A-A, A-Q, and Q-A* represent the four groups, respectively. (**F**) Fold change of CD1c$^+$ mDC proportions in each patient from groups of Q-Q, A-A, A-Q, Q-A was calculated and compared by ANOVA analysis ($P = 0.005$). The data are presented as mean ± SD. (**F**) Receiver operating characteristic curve was plotted and the area under the curve was obtained ($A = 0.64$, $P < 0.035$). Paired Student's t-test was used in (**A**–**C**).

causes T-cell immunosenescence[18,19] and our recent publication showing increased CD16 expression on monocytes after dexamethasone treatment in vivo and in vitro,[13] we expected that age or systemic prednisone treatment would have some associations with the levels of CD1c$^+$ mDC proportions. However, we were unable to find any association with age or treatment. Moreover, there was no significant association between proportions of CD1c$^+$ mDCs and the various individual etiologic or anatomic diagnoses associated with uveitis. This could be due to the small number of patients within each individual diagnostic subgroup or possibly a common autoimmune mechanism shared by these different causes and locations of noninfectious uveitis.

In the longitudinal data analysis, proportions of CD1c$^+$ mDC closely correlated with disease activity. Of our 44 patients with longitudinal CD1c data, only 4 quiescent patients activated during the blood sampling period as compared to 14 active patients who became quiescent. This is good for our patients who are undergoing treatment for uveitis; however, it gave us a small number of patients who activated after becoming quiescent for analysis. In addition, there were several apparent exceptions to this association in the data: one quiescent patient remained quiescent despite an increase in CD1c$^+$ mDC proportion from 45% to 75%, and two active patients remained active despite a decrease in CD1c$^+$ mDC proportion from 87% to 66% and 65% to 44%. Further clinical follow-up ultimately validated the overall correlation between CD1c$^+$ mDC proportion and disease activity: the quiescent patient suffered disease recurrence 7 weeks after the second blood draw and the two active patients became quiescent 1 and 3 weeks after the second blood draw. These findings suggest that CD1c$^+$ mDCs may predict disease activity in the near future, which led us to further investigate the levels of CD1c$^+$ mDCs in disease recurrence.
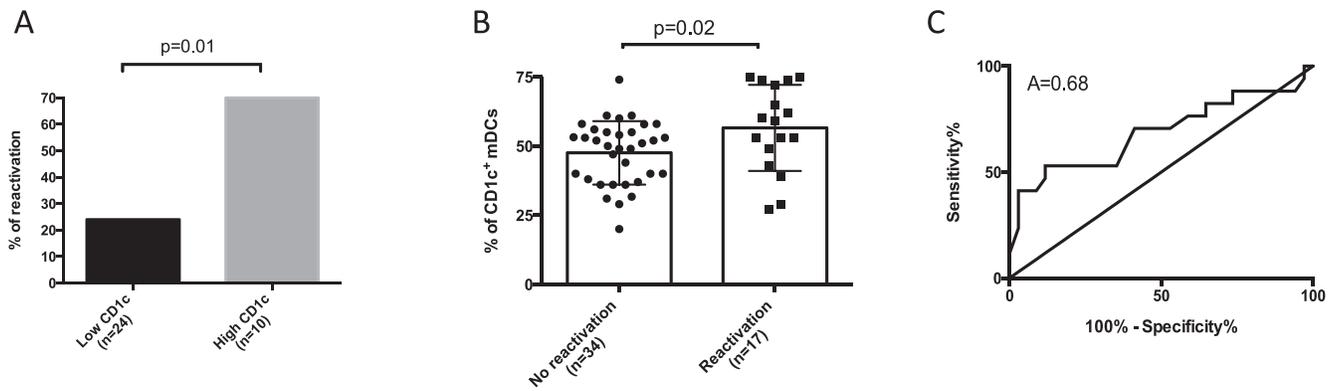
FIGURE 5. High proportions of CD1c$^+$ mDCs in quiescent patients were associated with future disease reactivation. The level of CD1c$^+$ mDCs at 60% was set as the cutoff, which was the average proportional level in active uveitis patients. (A) Patients ($n = 34$) were divided into low levels of CD1c proportion ($\leq$60% of CD1c$^+$ mDCs) and high levels of CD1c proportion (>60% of CD1c$^+$ mDCs) groups (low CD1c versus high CD1c). Numbers of quiescent patients who further developed disease reactivation were calculated in both groups. Six of 24 patients had disease reactivation (25%) in low CD1c proportion group, and 7 of 10 patients (70%) had disease recurrence in high CD1c proportion group. (B) CD1c$^+$ mDCs proportions were compared in the groups of quiescent patients with or without disease reactivation (no reactivation versus reactivation). The data are presented as mean $\pm$ SD. (C) Receiver operating characteristic curve was plotted and the area under the curve was obtained ($A = 0.68$, $P < 0.03$). Unpaired Student's *t*-test was used in (A, B).

Although most quiescent patients had low proportions of CD1c$^+$ mDCs, there was still a relatively small population of quiescent patients (26%) that maintained high CD1c$^+$ mDC proportions in our cohort as defined by the average proportion of CD1c$^+$ mDCs in active uveitis patients (>60% of CD1c$^+$ mDCs). These quiescent patients with high CD1c$^+$ mDC proportions had significantly higher rate of subsequent disease reactivation as compared to quiescent patients with low CD1c$^+$ mDC proportions (reactivation rate in high versus low CD1c groups: 70% vs. 25%). However, we were unable to find a correlation between CD1c$^+$ mDC proportions and time to reactivation in clinically quiescent patients owing to the lack of a consistent follow-up schedule for all patients. In addition, we have previously found that the proportions of CD1c^{hi} mDC subpopulation in total CD1c$^+$ mDCs are associated with uveitis disease activity.[9] Although the current data of CD1c$^+$ mDCs are consistent with the published data, we were unable to correlate the proportions of CD1c^{hi} mDC subpopulation with disease reactivation. Additionally, we showed in vitro that MoDCs with low CD1c$^+$ mDC proportions were associated with their impaired function in assisting allogeneic T-cell proliferation. This observation, which is consistent with our previously published data that MoDCs with high CD1c$^+$ expression levels are correlated with high HLADR expression

and are partially regulated by p38-MAPK pathway,[1] suggests a possible explanation for the role of CD1c$^+$ mDCs in the pathophysiology of noninfectious uveitis and a potential therapeutic target.

Receiver operating characteristic analysis showed that CD1c$^+$ mDCs had an AUC of 0.68 with low sensitivity (44%) but high specificity (90%) for disease reactivation at the cutoff level of 60% of CD1c$^+$ mDCs. This cutoff corresponded to the average CD1c$^+$ mDC proportion in active uveitis patients in our cohort. The high specificity of this test means that the likelihood of a false-positive result is low and a true-positive result is high. This is clinically valuable as it allows physicians to reserve prolonged immunosuppressive therapy for only those patients at highest risk for disease reactivation. On the other hand, the low sensitivity of this test means that some patients at risk for reactivation may have erroneously negative results. Though interesting, it is apparent that our study is a preliminary investigation and that prospective studies with larger sample sizes and more rigorous controls are needed to validate CD1c$^+$ mDCs as a biomarker for uveitis activity and reactivation.

Several limitations exist in this study. Firstly, as we mentioned above, this was a single-center investigation with 89 patients and 111 healthy controls, which may limit the
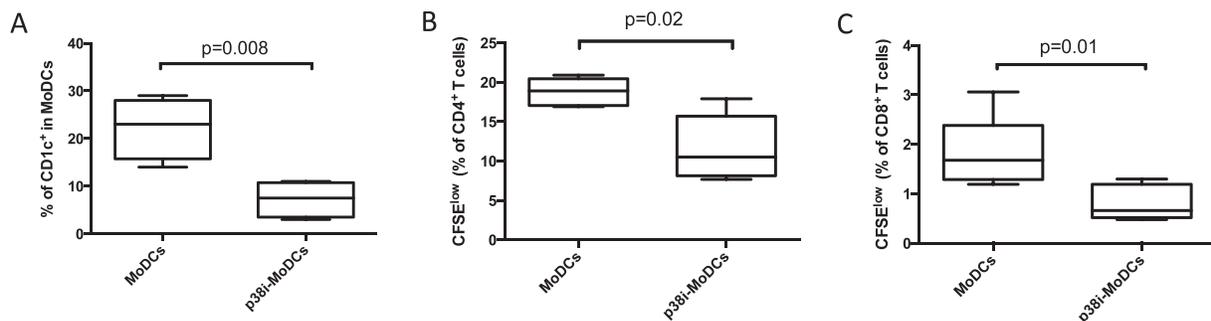

FIGURE 6. Monocyte-derived dendritic cells with low CD1c$^+$ proportions were associated with impaired allogeneic T-cell proliferation. Monocyte-derived dendritic cells were induced with or without treatment of p38 MAPK inhibitor in vitro. (A) Proportions of CD1c$^+$ MoDCs were measured by flow cytometry ($n = 4$). Monocyte-derived dendritic cells with or without p38 MAPK inhibitor treatment (p38i-MoDCs versus MoDCs) cocultured with allogeneic CD4$^+$ (B; $n = 5$) or CD8$^+$ T cells (C; $n = 5$) for 5 days, respectively. T-cell proliferation was measured by the dilution of CFSE on flow cytometry. The data are presented as mean $\pm$ SD. Paired Student's *t*-test was used for statistical analysis.

generalizability of our results. Secondly, we were unable to follow up patients from the beginning of their treatment course, since all the patients were referred from different local clinics to NEI. Thirdly, while we were able to generate longitudinal data for patients, these patients were not seen at regular intervals and thus changes in disease course in the interim could be missed. Fourthly, other factors may affect the CD1c+ mDC proportions, such as smoking, diet, and body mass index, which were not controlled in this study. Fifthly, further mechanistic studies are required to understand the cause and effect of increased CD1c+ mDC proportion in uveitis, such as overexpression or knockout CD1c expression on MoDCs.

In conclusion, the proportion of CD1c+ mDCs reflects disease activity and was associated with high risk of disease reactivation in some clinically quiescent patients with noninfectious uveitis. This carries significant potential for improving the clinical management of noninfectious uveitis.

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