Aberrant distribution and function of plasmacytoid dendritic cells in patients with ankylosing spondylitis are associated with unfolded protein response

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Abstract

Although human leucocyte antigen (HLA)-B27 is strongly associated with ankylosing spondylitis (AS), the association of unfolded protein response (UPR) induced by HLA-B27 misfolding in AS remains controversial. Since dendritic cells (DCs) are crucial in induction of AS in HLA-B27-transgenic rats, and plasmacytoid DCs (pDCs) belong to one type of DCs, we here aim to study the relevance of pDCs and UPR in AS. Peripheral pDCs were isolated from 27 HLA-B27(+) AS patients and 37 controls. The bone marrow (BM) and synovium of inflamed hips from AS patients and controls were obtained. We found a significantly higher frequency of pDCs in the peripheral blood, BM, or inflamed synovium of hips, which is associated with the enhanced expression of pDC trafficking molecules, CCR6 and CCL20 in the synovium of AS patients. Functional analysis further revealed that several inflammatory cytokines, including TNFα, IL-6, and IL-23, secreted by pDCs were significantly increased in AS patients.
patients as compared with those in controls. Remarkably, protein kinase RNA-like endoplasmic reticulum kinase (PERK) pathway in UPR was up-regulated in pDCs of AS patients. Notably, PERK inhibitor treatment significantly inhibited the enhanced cytokine production by pDCs of AS patients. Further, the extent of PERK activation was significantly associated with the increased disease severity of AS patients. Our data uncover the aberrant distribution and function of pDCs in AS patients. The up-regulated PERK pathway in UPR of pDCs not only contributes to enhanced cytokine production of pDCs, but also is associated with increased disease activity of AS patients.

**KEYWORDS**
ankylosing spondylitis, cytokine, HLA-B27, plasmacytoid dendritic cells, unfolded protein response

**1 | INTRODUCTION**

Ankylosing spondylitis (AS) is a prototypic type of spondyloarthropathy (SPA) characterized by inflammatory spondylitis, peripheral arthritis, and enthesitis. Typically, it occurs in young adult males and has a strong association with human leukocyte antigen (HLA)-B27. Although the genetic link between HLA-B27 and AS has been identified for more than 40 years, how it contributes to AS pathogenesis remains inconclusive. Prior mechanistic studies using HLA-B27-transgenic (B27-TG) rat model have suggested that upregulation of endoplasmic reticulum (ER) stress triggered by misfolding of HLA-B27 heavy chain in macrophages or dendritic cells is involved in the development of SPA through the interleukin (IL)-23/IL-17 axis. However, whether unfolded protein response (UPR) is relevant to AS patients is controversial, because macrophages from AS patients exhibited greater IL-23 production in response to lipopolysaccharide but had no significant UPR induction. Further studies are required to determine whether certain immune cell types or tissue-resident cells show evidence of UPR activation to drive the inflammation and pathogenesis of AS.

Dendritic cells (DCs), which are essential for bridging innate and adaptive immunity are strongly linked with the induction and maintenance of SPA. DCs can be commonly divided into two classes: plasmacytoid dendritic cells (pDCs) and conventional dendritic cells (cDCs). While cDCs can be further subdivided into three populations based upon the expression of surface markers: CD1c, CD16, or blood dendritic cell antigen (BDCA)-3. pDCs are characterized by the expression of those positive markers: CD123, CD303 (BDCA-2), and CD304 (BDCA-4). pDCs are a unique population of bone marrow (BM)-derived immune cells with plasma cell-like morphology that can produce large amounts of type I/III interferon (IFN) and inflammatory cytokines upon activation by pathogen-derived nucleic acid. Compared with cDCs which are professional antigen presenting cells, pDCs are less efficient in priming naïve T cells but have enhanced ability to present endogenous antigen for inducing tolerance. In B27-TG rat model, DCs exhibit increased apoptotic cell death, loss of self-tolerance, impaired cytoskeletal dynamics, and more pathological ability to induce pathogenic IL-17 producing cells.

In addition, overexpression of genes involved in ER stress has been identified in splenic CD103<sup>+</sup> cDCs of B27-TG rats. However, parallel studies on the role of DCs in AS patients were rare, most of which focused on DCs generated from monocytes cultured with granulocyte-macrophage colony-stimulating factor and IL-4. Nevertheless, the relevance of human DCs derived from cultured monocytes to the authentic AS pathophysiology is unclear. Although it has been reported that freshly isolated CD1c<sup>+</sup> cDCs from AS patients do not display increased UPR response as compared with those from healthy controls, the relationship of freshly isolated pDCs and UPR in human AS pathogenesis has not been demonstrated. In this regard, we sought to examine the function of pDCs of AS patients and explore whether UPR activation in pDCs contributes to the pathogenesis of AS.

**2 | MATERIALS AND METHODS**

**2.1 | Subjects**

Peripheral blood was obtained from AS patients (n = 27) who met the modified New York criteria or healthy controls (n = 37). Serologic tests (HLA-B27, C-reactive protein or erythrocyte sedimentation rate), clinical assessments, and radiographic assessments on AS patients were obtained on the same day. Detailed in scoring systems and the demographic characteristics were shown in Supporting Information and Table S1. For tissue study, we obtained BM specimens of lumbar spines from AS patients with spinal ankylosis who had undergone spinal osteotomy (A1, A2, and A3) and from normal controls acquired from US Biomax Company (C1, C2, and C3) (Table S2). Synovial tissues of hips were obtained from AS patients who had undergone total hip replacement (A4, A5, and A6) and non-AS controls who received total hip replacement due to femoral neck fracture after traffic accidents (C4, C5, and C6) (Table S3). All donors provided written informed consent before sampling in accordance with the Declaration of Helsinki. The study protocol was approved by the Research Ethics Committees of Taipei Tzu Chi Hospital, Taipei Veteran General Hospital and Academia Sinica, Taiwan.
2.2 Purification of pDCs from peripheral blood and culture of pDCs

Peripheral blood mononuclear cells (PBMCs) were obtained from whole blood by centrifugation over a Ficoll-Hypaque density gradient (GE Healthcare). pDCs were isolated and purified by magnetic cell separation from PBMCs using anti-BDCA-2+ Microbeads (Miltenyi Biotec). The purity of BDCA-2+ pDCs was about 90%, as confirmed by flow cytometry (detailed in Supporting Information and Figure S1). Next, $5 \times 10^4$ freshly isolated pDCs were resuspended in $150 \mu L$ RPMI-1640 medium plus 1% penicillin/streptomycin, 1% sodium pyruvate, and 10% fetal bovine serum (all from Gibco). The cells were seeded in 96-well plate, supplemented with 10 ng/mL IL-3 to prevent cell apoptosis (PeproTech), with/without 1 mM class A cytosine phosphatidyl guanine oligodeoxynucleotide (CpG ODN) 2216 (InvivoGen), or with/without 0.4 nM protein kinase RNA-like endoplasmic reticulum kinase (PERK) inhibitor (GSK2606414, Merck Millipore), and incubated for 24 hours at 37°C and 5% CO₂.

2.3 Cytokine release

Production of tumor necrosis factor (TNF) α, IL-6, IL-1β, and IL-23 (p19) by pDCs in culture supernatants was examined 24 hours after incubation. Cytokines were detected using a Milliplex kit (Merck Millipore) according to manufacturer’s instructions. Production of IFN-α and IFN-β by pDCs in culture supernatants with or without CpG ODN 2216 treatment was determined 24 hours after incubation by enzyme-linked immunosorbent assay kits (PBL Interferon Source or R&D, respectively) according to manufacturer’s instructions.

2.4 Immunohistochemistry (IHC) staining

Sections of BM or synovium specimens were incubated with goat anti-BDCA-2 antibody or goat anti-chemokine ligand (CCL) 20 antibody (both from R&D), followed by incubation with secondary antibody, biotinylated anti-goat-IgG (Southern Biotech). Then sections

**FIGURE 1**  Increased frequency of pDCs in PBMCs and BM of AS patients, as compared with those of normal controls. A, Comparison of the proportion of peripheral blood pDCs over total PBMCs between normal controls (n = 27) and AS patients (n = 37). B, RT-QPCR showing the mRNA levels of FLT3 in pDCs of normal controls and AS patients. C, Representative IHC staining with anti-BDCA-2 antibody (brown stains) against pDCs in the BM sections of normal controls (C1, C2, and C3; n = 3) and AS patients (A1, A2, and A3; n = 3). Scale bar = 100 μm. Inset represents high magnification from the boxed area. D, Enumeration of pDCs in the BM was determined by counting cells on microphotographs obtained from five randomly selected HPFs (0.5 mm²) per individual. The mean values of five HPFs were shown. Data represent the mean ± SEM. *P < .05, and **P < .01 denote a significant difference by an unpaired, two-tailed Student’s t test.
were incubated with Elite ABC-HRP kit (Vector Labs). The stains were visualized with chromogen 3, 3'-diaminobenzidine and nuclear was counterstained with hematoxylin (both from Thermo Fisher Scientific). The number of pDCs was counted in 0.05 mm² on microphotographs obtained from five randomly selected high-power microscopic field (HPF). Mean values of fives HPFs were recorded individually.

2.5 | Reverse transcription quantitative-polymerase chain reaction (RT-QPCR)

Detailed in RT-QPCR were shown in Supporting Information.

2.6 | Statistical analysis

Data are the mean ± SEM. Comparisons between two groups were assessed using an unpaired, two-tailed Student’s t test. An assessment of the normality of data was conducted by performing Shapiro-Wilk test, which confirmed that the dataset was modeled by the normal distribution. Wilcoxon matched-paired signed rank test was performed to compare the difference in cytokine levels before and after PERK inhibitor treatment in paired samples. Correlations between variables were determined with the Spearman’s rank correlation test. P < .05 was considered statistically significant. Data were analyzed by JMP 5.0.1 or Prim 6.01.

3 | RESULTS

3.1 | Increased frequency of pDCs in PBMCs and BM of HLA-B27(+) AS patients

We examined the systemic and local frequency of pDCs in AS patients. The proportion of pDCs in PBMCs were significantly elevated in AS patients, as compared with those from normal controls (0.21 ± 0.02% and 0.14 ± 0.01%; P < .01) (Figure 1A). Since FMS-like...
tyrosine kinase (FLT) 3 ligand-mediated FLT3 signaling is critical for pDCs development and homeostasis,12 we checked the expression of FLT3 in the pDCs. Our result showed that FLT3 levels are elevated in pDCs of AS patients (Figure 1B). We next examined the pDC contents in the BM as pDCs develop in BM.12 IHC staining with anti-BDCA-2 antibody showed the enrichment of pDCs in the BM of AS patients, as compared with those from normal controls (21.20 ± 5.54/HPF and 4.40 ± 0.702/HPF; \( P < .05 \)) (Figure 1C,D). These results demonstrated the increased frequency of pDCs in PBMCs and BM of AS patients.

3.2 | Aberrant distribution of pDCs to the inflamed synovium of hips in HLA-B27(+) AS patients may be mediated through the enhanced expression of trafficking molecules

It is also likely that increased pDCs in peripheral tissues may be resulted from the abnormal migration of pDCs to the site of inflammation.12 Since hip is a common inflammatory site in 24% to 36% of AS patients,20 we assessed the distribution of pDCs in synovial tissues of hips. The recruitments of pDCs in inflamed hips of AS patients were significantly enhanced, compared with those of non-AS controls (7.33 ± 0.44/HPF and 0.267 ± 0.17/HPF; \( P < .001 \)) (Figure 2A,B). It has been shown that chemokine receptor (CCR) 6 and its ligand CCL20 mediate the recruitment of pDCs into the inflamed epithelia.21 Thus, to determine the possible mechanism causing increased attraction of pDCs in inflamed synovial tissues of AS, we checked the expression of CCR6 in pDCs and CCL20 in the synovium of AS patients. Our result showed that pDCs of AS patients displayed higher expression levels of CCR6 than those of normal controls (Figure 2C). In addition, the upregulated expression of CCL20 in the synovium of AS was observed, most likely among synoviocytes, vascular endothelial cells or infiltrating mononuclear cells, as compared with those of non-AS controls (Figure 2D). These results suggest that the enhancement of CCR6-CCL20 may mediate the aberrant trafficking of pDCs to inflamed hips of AS patients.

3.3 | Increased production of inflammatory cytokines by pDCs of HLA-B27(+) AS patients

Next, in order to determine whether pDCs from AS patients may induce or sustain inflammation, we examined their cytokine production. Since several cytokines, including TNFα, IL-6, IL-1β, and IL-23, have been shown to be associated with AS,22 we found that unstimulated pDCs of AS patients produced significantly higher levels of inflammatory cytokines (TNFα, IL-6, and IL-23) than those of controls (Figure 3A-D). Both naïve pDCs from AS patients and controls did not secret measurable amount of type I IFN (IFN-α/β) (data not shown). However, we found that there was no significant difference in IFN-α or IFN-β production in CpG-stimulated pDCs between AS patients and normal controls (Figure 3E,F). Collectively, these data show the enhanced production of inflammatory cytokines by pDCs in AS patients.

3.4 | UPR activation contributes to enhanced production of inflammatory cytokines in AS pDCs

We then investigated the expression of genes involved in UPR and assessed their correlation with the enhanced cytokine productions by

**FIGURE 3**  Increased production of inflammatory cytokines by pDCs of HLA-B27(+) AS patients. A-D, production of cytokines by cultured unstimulated pDCs isolated from normal controls (n = 27) and AS patients (n = 37). Concentrations (pg/mL) of TNFα (A), IL-6 (B), IL-1β (C), and IL-23 (D) in cultured medium were shown. E and F, Concentrations of IFN-α and IFN-β secreted by CpG-stimulated pDCs of normal controls (n = 11) and AS patients (n = 13). Levels of IFN-α (E) and IFN-β (F) in cultured medium were shown. Data are the mean ± SEM. *\( P < .05 \), and ***\( P < .001 \) denote a significant difference by an unpaired, two-tailed Student’s t test.
pDCs in HLA-B27(+) AS patients. We examined the expression of binding immunoglobulin protein (BIP), the global marker of UPR, and genes involved in UPR pathways including PERK/activating transcription factor 4 (ATF4)/CCAAT-enhancer-binding protein homologous protein (CHOP), inositol-requiring 1 (IRE1)/spliced X-box binding protein 1 (sXBP1), and activating transcription factor 6 (ATF6). We found that the mRNA levels of BIP and PERK/ATF4/CHOP were all significantly up-regulated in pDCs of AS patients (Figure 4A-D), whereas IRE1/sXBP1 and ATF6 mRNA levels in pDCs did not differ between AS patients and controls (Figure 4E-G). Then, to further determine whether the enhancement of PERK pathway in UPR contributes to the increased production of inflammatory cytokines in pDCs of AS patients, we examined the changes of cytokine levels secreted by pDCs after treatment with PERK inhibitor. Remarkably, treatment with PERK inhibitor significantly reduced the amounts of TNFα, IL-6, and IL-23 secreted by unstimulated pDCs of AS patients (Figure 4H-K). Altogether, these findings suggest that PERK/ATF4/CHOP pathway in UPR may contribute to the enhanced production of inflammatory cytokines in pDCs of HLA-B27(+) AS patients.

3.5 The extent of PERK activation in pDCs is positively correlated with the elevated disease activity score of AS patients

We then examined whether aberrant distribution and function of pDCs are associated with clinical parameters of AS patients. It is noted that the extent of PERK activation in pDCs is positively correlated with the elevated disease activity score (BASDAI) in AS patients (Table 1). However, the percentage of pDCs in PBMCs did not correlate with systemic inflammatory parameters (CRP/ESR), disease activity (BASDAI) or radiographic severity (mSASSS or BASRI-total) in AS patients (Table S4). Together, our results show that the up-regulated PERK pathway not only contributes to the enhanced inflammatory response in pDCs of AS patients. 

**FIGURE 4** UPR activation contributes to the enhanced inflammatory cytokine production in pDCs of HLA-B7(+) AS patients. A-G, RT-QPCR showing the mRNA levels of genes involved in UPR in unstimulated pDCs of controls (n = 27) and AS patients (n = 37). Data represent the mean ± SEM. *P < .05, **P < .01, and ***P < .001 denote a significant difference by an unpaired, two-tailed Student’s t test. H-K, Levels of cytokines (pg/mL) produced by cultured unstimulated pDCs of AS patients with or without the treatment of PERK inhibitor. Levels of TNFα (H), IL-6 (I), and IL-23 (K) were inhibited after PERK inhibitor treatment. Wilcoxon matched-paired signed rank test was performed to compare the changes of cytokine levels secreted by pDCs before and after PERK inhibitor treatment in paired samples (n = 6) and *P < .05 denote a significant difference.
TABLE 1  Correlation of PERK pathway activation in pDCs with clinical parameters, disease activity, and radiographic severity

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<td>pDCs percentage in PBMCs (%)</td>
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Note: r = Spearman’s correlation coefficient. Abbreviations: BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASRI-total, Bath Ankylosing Spondylitis Radiology Index-total score; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; mSASSS, modified Stoke Ankylosing Spondylitis Spinal Score. *P value is determined by the Spearman’s rank correlation test and P < .05 is statistically significant.

cytokine production in pDCs of AS patients, but also is also associated with increased disease activity of AS patients.

4 | DISCUSSION

To date, the roles of pDCs in autoimmune diseases have been studied in patients with systemic lupus erythematosus, type I diabetes, psoriasis, and autoimmune thyroid disease, but little is known about their involvement in the pathogenesis of AS patients, majorly owing to the fact that pDCs are a rare population in human peripheral blood, accounting for approximately 0.2% to 0.5% of PBMCs. Here, we identified a significantly higher frequency of pDCs in the peripheral blood, BM or inflamed synovium of hips in HLA-B27(+) AS patients, possibly via the enhanced CCR6-CCL20 mediated trafficking. Of note, we uncover the altered production of inflammatory cytokines, including TNFα, IL-6, and IL-23, by pDCs in AS patients, in which the upregulated UPR of PERK signaling pathway may be involved.

It has been shown that misfolding of HLA-B27 heavy chain could induce the activation of CHOP and XBP1 in the macrophages of B27-TG rats. However, macrophages in AS patients do not show the enhanced UPR; thus, whether UPR is the culprit in the pathogenesis of AS remains controversial. The increased expression of BIP has been found in macrophages or mononuclear cells in synovial fluid of SPA patients. Therefore, it has been hypothesized that certain cell types might be more capable to handle the increased ER load induced by UPR to adapt the chronic ER stress. Indeed, HLA-B27 misfolding-related UPR could be found in BM-derived macrophages, but not in splenocytes or thymus in B27-TG rats. Further, our recent findings demonstrated that HLA-B27 misfolding activates IRE1/sXBP1 in mesenchymal stem cells (MSCs) derived from the entheseal tissues of spinal ankylosis of AS patients (AS-derived MSCs), suggesting that UPR is as well present in the stromal microenvironment of HLA-B27(+) AS patients. Here, our data demonstrate that pDCs of HLA-B27(+) AS patients also elicit UPR. Future studies are warranted to clarify how the UPR only occurs in specific cell types.

Moreover, we have found that the enhanced production of inflammatory cytokines in pDCs and demonstrated their association with the UPR activation in HLA-B27(+) AS patients. Among the three major UPR pathways, we firstly uncover the importance of PERK/ATF4/CHOP pathway in regulating the inflammatory phenotype of pDCs in AS patients. Interestingly, this PERK pathway was also activated in AS-derived MSCs but did not contribute to the abnormal osteogenesis. Rather, it is the activation of IRE1/sXBP1 in UPR that promotes the abnormal osteogenesis. Several reports have demonstrated how PERK/ATF4/CHOP pathway influences the production of TNFα, IL-6, and IL-23 in certain circumstances. In mDCs of healthy donors, CHOP was found to bind to the promoter of IL-23 (p19). Besides, in human intestine epithelial cell lines, CHOP could enhance nuclear factor-κB (NF-κB) signaling to induce the production of TNFα/IL-6. PERK also preferentially affects the negative regulator of NF-κB, leading to enhanced activation/translocation of NF-κB into the nucleus, thereby inducing the expression of TNFα and IL-6 in mice. Furthermore, ATF4 binds to the cAMP response element in the promoter of Il-6 in macrophages of mice. These data showed how PERK/ATF4/CHOP pathway regulates the production of TNFα, IL-6, and IL-23 in distinct cell types. Although the detailed mechanisms of how PERK/ATF4/CHOP pathway regulates the production of TNFα, IL-6, and IL-23 in pDCs of HLA-B27(+) AS patients required further study, our results indicated that upregulated PERK signaling pathway in UPR may connect how HLA-B27 contributes to the inflammation in pDCs of AS patients.

Furthermore, pDCs are best known for their extraordinary ability to produce type I IFN in response to RNA/DNA viruses through toll-like receptor 7/9, respectively, thereby able to defense against viral infection. In SLE or psoriasis, pDCs are activated by circulating immune complexes comprising self-DNA released from injured tissues and antibodies to DNA, resulting in type I IFNs production to induce activation of cDCs and subsequent stimulation of autoreactive T cells. In addition to producing large amount of IFN-α, pDCs could also produce IFN-β at lower levels. However, the role of type I IFN in AS pathogenesis has not been well established. In B27-TG rats, additional treatment of IFN-γ, a type II IFN, in BM-derived macrophages upregulated the expressions of HLA-B27, BIP, CHOP, and sXBP1, displaying a prominent IFN-responsive genes signature from microarray. On the other hand, downregulation of IFN has been found in splenic DCs from B27-TG rats or monocyte-derived macrophages from AS patients. However, our data reveal that the production of IFN-α/β is undetectable at basal levels and comparable in CpG ODN 2216-stimulated pDCs from AS patients and controls. These results suggested that type I IFN produced by pDCs, likely triggered by self-DNA, may not be the significant player in the pathology of AS.
Regarding to tissues distribution, little is known about the dynamics of pDCs accumulation in the inflamed hips of AS patients. Here, we found that pDCs of AS patients express higher CCR6 tissue homing marker, and express enhanced level of CCL20 in the synovium, which may contribute to the increased frequency of pDCs in the inflamed hips of AS patients. In chronic arthritis in mice, it has been demonstrated that TNFα, IL-17, and IL-1p are the potent inducers for the production of CCL20 in synovioocytes. As we did not measure their expression levels in in situ milieu, the enhanced expression of TNFα, IL-17, and IL-1p has been reported in inflamed joints of AS in previous studies. In addition, given that transforming growth factor beta, IL-6, IL-17, and IL-23 have been reported to regulate CCR6 expression, it remains to be clarified whether these cytokines serve as the upstream regulator for CCR6 expression in pDCs of AS patients. Finally, since pDCs can prime CD8+ T cells or Th17 cells differentiation, the pivotal role of pDCs for promoting the Th17-driven inflammatory immune responses in inflamed hips of AS patients would be worthy of exploration in the future.

In conclusion, our results demonstrate higher frequencies of pDCs in peripheral blood, BM, or inflamed synovium of hips, which is linked with the enhanced CCR6-CCL20 mediated trafficking of HLA-B27(+) AS patients. Moreover, the increased frequency of pDCs in AS patients might be related to the enhanced expression of FLT3, although whether the elevated expression of FLT3 in pDCs of AS patients is associated with the activation of UPR or cytokine stimulation deserves further investigation. Notably, we also found the enhanced production of several inflammatory cytokines by pDCs of HLA-B27(+) AS patients. Of interest, the up-regulated PERK signaling pathway in UPR not only contributes to enhanced cytokine production by pDCs, but also is associated with increased disease activity of AS. These results suggest the pathological role of pDCs in AS. Further understanding of the interaction of pDCs with IL-23 responsive cells or priming of Th17 cells differentiation may shed light on our understanding of the pathogenesis of AS and on the development of novel therapeutics for AS patients in the future.

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CONFLICT OF INTEREST
The authors declare no potential conflict of interest.

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REFERENCES
19. Axten JM, Medina JR, Feng Y, Shu A, Romeril SP, Grant SW, et al. Discovery of 7-methyl-5-(1-[[3-(trifluoromethyl)phenyl]acetyl]-2,3-dihydro-1H-indol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of


SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.