Immunomodulatory effects of bexarotene on tumor-associated macrophages in patients with mycosis fungoides.

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Introduction and backgrounds

The immunological background of mycosis fungoides (MF) differs with each tumor stage. As we previously reported, in the early stage, the cancer stroma contains a dense deposition of periostin (POSTN), whereas in the advanced stage, IL-4 expression rather than POSTN deposition is prominent. Since POSTN modulates cell functions including proinflammatory chemokine production, we hypothesized that the immunological background of MF might be an inflammatory condition in early stages and a steady state in late stages.

Early stage MF: POSTN>IL-4 Advanced stage MF: POSTN<IL-4

A series of chemokines: CD163+ Macrophages stimulated by POSTN; microarray analysis

A series of chemokines: CD163+ macrophages stimulated by IL-4; microarray analysis

Materials and methods

Etics

This study was approved by the ethics committee of Tohoku University Graduate School of Medicine, Sendai, Japan (2016-1-650, 2017-1-428, 2017-1-429,). All patients were given informed consent.

Generation of M2 macrophages from PBMC: for the investigation of advanced CTCL

CD14⁺ cells were isolated from the peripheral blood mononuclear cells (PBMCs) of healthy donors using MACS beads (CD14 microbeads, Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. CD14⁺ monocytes (2×10^5 /ml) were cultured in complete medium containing 100 ng/ml of recombinant human M-CSF for 5 days, followed by IL-4 with or without bexarotene.

Results

Fig. 1: Immunofluorescence staining of advanced CTCL

Immunofluorescence staining of advanced for CCL22 (green), CD163 (red) and DAPI (blue, nucleus). A merged image is also shown. Merged green and red stain yellow. Scale bars, 50 mm. Representative specimens from 4 cases are shown.

Immunofluorescence staining of advanced for CCL17 (green), CD163 (red) and DAPI (blue, nucleus). A merged image is also shown. Merged green and red stain yellow. Scale bars, 50 mm. Representative specimens from 4 cases are shown.

Fig. 2: Bexarotene reduced the mRNA expression of Th2 chemokines from CD163+ M2 macrophages

M2 macrophages were induced from PBMCs from healthy donors, as described in Materials and Methods. On the fifth day, the monocyte-derived M2 macrophages were treated with IL-4 (20 ng/ml) together with graded bexarotene. Six hours after stimulation, total RNA was recovered from macrophages and amplified, labeled, and analyzed. Quantitative real-time PCR for validation of the DNA microarray results was conducted to determine the number of copies of cDNA for each chemokine, and the mRNA expression levels relative to the mRNA expression levels of IL-4-stimulated cells were calculated for each gene and time point after normalization against GAPDH using the $\Delta\Delta Ct$ method.

Fig. 3: Bexarotene reduced the production of Th2 chemokines from CD163+ M2 macrophages

The culture supernatant from M2 macrophages was harvested as described in Materials and Methods and measured by ELISA. Data from each donor were obtained from triplicate assays, and the mean $\pm$ SD was calculated. Average data from at least three independent experiments are shown. Asterisk: $p < 0.05$, by Student's t-test; n.s., not significant.

Fig. 4: Serum levels of CCL22 in patients with CTCL: bexarotene reduced serum CCL22 in bexarotene responders

The serum from CTCL patients was measured by ELISA. Data from each donor were obtained from triplicate assays, and the mean $\pm$ SD was calculated. Asterisk: $p < 0.05$, by Student's t-test; n.s., not significant.

SUMMARY

1. CD163+ TAMs in advanced CTCL expressed CCL22, but merely expressed CCL17.
2. Bexarotene decreased the mRNA expression of Th2 chemokines (CCL17, CCL22) and increased Th1 chemokines (CXCL10, CXCL11) on monocytes-derived CD163+ macrophages in vitro.
3. Bexarotene did not effect on the activation of CD163+ macrophages, but decreased the production of CCL22 from CD163+ macrophages in vitro.
4. Serum CCL22 was increased in parallel with the progression of CTCL.
5. Serum levels of CCL22 was decreased in CTCL patients who responded to bexarotene.

References

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