CIRCULATING REGULATORY T-CELL NUMBER DOES NOT PREDICT PROGNOSIS OF MONOCLONAL GAMMOPATHIES OF UNCERTAIN SIGNIFICANCE

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BACKGROUND

FOXP3-expressing regulatory T-cells (Tregs), which suppress aberrant immune response against self-antigens, also suppress anti-tumor immune response. It has been shown that there is an increased proportion of Tregs in several different human malignancies, although the actual mechanism remains unclear. The research aims to explore the relationship between the number of Tregs and a predict prognosis in particular hematological diseases as monoclonal gammopathies of uncertain significance (MGUS).

METHODS

Clinical, biological and laboratory features of 56 patients diagnosed with MGUS were retrospectively evaluated aiming at verify whether circulating Tregs number detected at diagnosis could have prognostic significance in terms of ability to predict progression to overt MM. Tregs were evaluated by means of flow cytometry (FACSCanto ™ II Becton Dickinson, BD, San Jose, CA, USA) using a combination of four conjugated monoclonal antibodies (MoAbs): CD127-PE/CD4-PerCP/CD25)-Pe-Cy7/CD45-APC Cy7 (BD). A stain, lyse no-wash standard procedure was employed, as follows: incubation of 15 minutes of 100µL of peripheral blood sample with recommended amount of each MoAbs.

A minimum of 100,000 events for each sample were acquired and analyzed using FACS-Diva software (BD). Tregs express the immunophenotype $CD4^{+}$ $CD25^{high/+}CD127^{low/-}$.

At a mean follow-up of 85 months (range 77 - 93 months) 9 MGUS patients progressed to overt MM.

RESULTS

We found no differences between patients with stable MGUS and patients with MGUS progressed to MM in terms of clinical and laboratory features evaluated at diagnosis, but for the number of white blood cells (p 0.019). Notably, neither κ/λ free light chain ratio or MGUS risk categories according to the Mayo Clinic risk stratification model were found different in the two groups of patients, probably due to the limited number of cases in this cohort. Of interest, Tregs cell number, evaluated on lymphocyte total number and CD4+ gated T-lymphocytes as percentage and absolute number as well, was also found to have no statistical significance (Tab. 1). Finally, overall survival was not found different in the two groups of patients probably reflecting the great progresses made in the therapy of MM in the recent years and the short follow-up of the series

DISCUSSION

No prognostic relevance of Tregs was found in MGUS in our hands. Tregs have been shown to be increased in other cancers suggesting a critical role in disease onset and progression. This is not clearly established in gammopathies despite a role of T-cells imbalance in MM has been hypothesized since several years ago. Tregs have an essential role in the homeostasis of the immune system playing immunosuppressive activity. However, despite a general agreement on the functional efficiency of Tregs in monoclonal gammopathies, conflicting results have been reported so far by different groups with respect to their prognostic significance.

CONCLUSIONS

Despite Tregs are known to play a crucial role in controlling the homeostasis of immune system since 70ˢ, an agreement among researchers about how these cells have to be analyzed (i.e., total Tregs or subpopulations such as activated, resting or non-suppressive Tregs, FoxP3-expressing Tregs) has not reached so far. All above taken into account, the urgent need of standardization emerges so that comparable procedures are used in all laboratories, as in similar laboratory conditions when small size cell populations are studied (i.e., CD34+ cells and dendritic cells), in particular when antigens are expressed at low, or very low, density. In fact, only with stringent and well-defined processing and analyzing criteria, reproducible findings may be obtained in order to draw definitive and reliable conclusions.

Table 1

	All patients (no. 56)	Progressed to MM (no. 9)	Stable (no. 47)	p
Lymphocytes) % (range)	2.00 (0.3-4.4)	2.10 (1.10-3.80)	2.0 (0.30-4.40)	
Median Tregs (on Lymphocytes (/µL) (range)	34.69 (0.60-643.70)	53.70 (0.60-64.20)	34 (1.10-643.70)	ns
Median Tregs (on CD4+ lymphocytes) (%) (range)	4.70 (0.50-10)	5.45 (2.50-6.20)	4.55 (0.50-10)	ns
Median Tregs (on CD4+ lymphocytes) (/µL) (range)	35.35 (2-96.90)	52.40 (21.70-63.70)	33.30 (2-96.90)	ns