Monoclonal Gammopathies

CD45RA⁺ and CD45RO⁺ lymphocyte subpopulations in patients with monoclonal gammopathies: correlations with diagnosis, clinical stage and treatment status

Alterations in blood lymphocyte subsets may be involved in the development of overt myeloma. Naive (CD4⁺CD45RA⁺) and memory (CD4⁺CD45RO⁺) helper T-cell subsets are important effectors of immune T-cell regulation. We analyzed the distribution of these blood lymphocyte subpopulations in patients with monoclonal gammopathies, considering the type of disorder, clinical stage, and treatment status.

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Some authors have reported that peripheral blood CD4⁺ cells are significantly decreased in myeloma patients, and suggested that this could play a role in malignant transformation and myelomatous clone control.[1] However, there are two functionally different subsets of CD4⁺ cells: naive (CD45RA⁺) and memory (CD45RO⁺) helper T-cells.[2,3] The ratio of CD4⁺CD45RA⁺ to CD4⁺CD45RO⁺ cells is approximately 1:1 in healthy adults. The distribution of these subsets, rather than global CD4⁺ cell analysis, may be relevant in monoclonal gammopathies. We studied blood samples from 54 patients with MG (32 females, 22 males; median age 66 years), and 10 healthy controls. Of these 54 patients, 33 had multiple myeloma (MM), 6 had smoldering MM (SMM), 7 had MG of undetermined significance (MGUS) and 8 had Waldenström´s macroglobulinemia (WM). MM patients were subdivided in four groups: those at diagnosis (MM-D, n=9), those with an objective response (MM-OR, n=6), partial response (MM-PR, n=11), and those in whom treatment had failed or who had disease progression (MM-TF/DP, n=7).[4] None of the MM patients was in complete remission. Clinical stage, according to the Durie & Salmon classification, was also recorded. Conjugated monoclonal antibodies used in the study were: CD19 (FMC63, clone identification; IgG2a, isotype), CD3 (UCHT-1; IgG1), CD4 (RPA-T4; IgG1), CD8 (LT8; IgG1), CD45RA (F8-11-13; IgG1), and CD45RO (UCHL1; IgG2a). Double-color immunofluorescence protocols (fluorescein-FITC/phycoerythrin-PE) were designed to identify CD3/CD19, CD4/CD8, CD4/CD45RA, and CD4/CD45RO cells. An EPICS® XL-MCL flow cytometer (Coulter Corp., Miami, USA) was used. Results are expressed as positivity percentage among gated lymphoid cells, and absolute number (lymphocytes per microliter). Descriptive statistics, including means and standard deviations, are used to prssent the results. Student´s t test was used to compare means of data with a normal distribution, and Mann-Whitney´s U test was used in the other cases. Differences were considered statistically significant when $p<0.05$.

The percentages of CD4⁺ cells were similar in patients with MGUS and SMM, and in the control groups, while they were significantly lower in the MM and WM patients ($p=0.03$). The absolute numbers of CD4⁺ cells decreased gradually from the highest number in controls (1302±364/μL) to MGUS (924±283), SMM (854±222), WM (698±484), and, finally, the lowest in MM patients (600±339), with a $p<0.001$. However, these changes in CD4⁺ lymphocytes depended on a specific alteration in the CD4⁺CD45RA⁺ subpopulation. The percentage of the naive helper T-cell cells was higher in controls (20.7±6.3) and patients with MGUS and SMM (20.4± 8.0 and 18.6±4.6, respectively) than in MM and WM patients (11.3±9.0 and 11.0±7.7) ($p=0.001$). Analysis of absolute CD4⁺CD45RA⁺ cell counts showed a more marked decline from healthy subjects (606±260/μL) to MGUS and SMM patients

Table 1. Distribution of blood lymphocyte subsets (percentage and absolute cells×10⁶/L) between patients with monoclonal gammopathies, once myeloma patients were separated by treatment status and response (mean ±standard deviation). NS = not significant.

Lymphocyte subpopulations	*MGUS N=7*	*SMM N=6*	*MM-D N=9*	*MM-OR N=6*	*MM-PR N=11*	*MM-TF/P N=7*	*WM N=8*	*p value*
% CD3⁺	78.19±6.41	72.18±12.86	75.72± 9.57	70.48±14.69	79.57±10.14	76.31±6.27	71.26±17.08	NS
Absolute CD3⁺	1448±278	1394±375	1514±546	1081±708	1464±887	1016±550	1425±1039	NS
CD19⁺	6.4±2.25	4.83±4.61	7.49±6.01	6.58±6.1	5.44±7.43	3.58±3.09	7.72±10.85	NS
Absolute CD19⁺	120±51	105±123	152±134	96±90	118±232	53±52	216±482	NS
% CD4⁺	49.3+/ 9.8	43.9±3.5	44.0±10.3	33.3±13.4	31.8±9.6	36.6±15.6	37.4±11.8	0.038
Absolute CD4⁺	924±283	854±222	851±283	503±319	554±317	481±341	698±484	0.08
% CD4⁺CD45RA⁺	20.4±8.0	18.6±4.6	19.2±11.7	7.7±6.7	8.1±4.8	10.3 ±6.2	11.0±7.7	0.002
Absolute CD4⁺CD45RA⁺	376±135	355±94	369±228	115±104	146±125	151±139	203±165	0.002
% CD4⁺CD45RO⁺	28.46±6.21	19.7±5.47	22.14±8.86	25.33±9.67	23.64±5.75	25.91±10.56	25.21±11.76	NS
Absolute CD4⁺CD45RO⁺	535±186	386±148	543±415	376±224	407±208	329±211	476±358	NS
% CD8⁺	28.41±15.05	31.55±8.04	32.02±18.06	36.98±7.11	47.55±16.96	41.86±16.66	33.5±11.26	NS
Absolute CD8⁺	517±274	594±133	662±429	574±421	905±693	560±309	723±632	NS
CD4/CD8 ratio	2.47±1.71	1.48±0.48	2.01±1.32	0.93±0.42	0.81±0.49	1.09±0.77	1.19±	NS
CD4⁺CD45RA⁺/ CD4⁺CD45RO⁺ ratio	0.75±0.41	1.02±0.38	1.16±1.07	0.29±0.2	0.33±0.17	0.43±0.23	0.59±0.52	0.005

Table 2. Differences in analyzed blood lymphocyte subsets (percentage and absolute cells×10⁶/L) between patients who did not receive treatment for their plasma cell disorder and those who did (mean ±standard deviation).

Lymphocyte subset	*Untreated patients N=22*	*Treated patients N=32*	*p value*
% CD3⁺	75.09±11.41	75.57±11.42	NS
Absolute CD3⁺	1551±441	1207±800	0.010
% CD19⁺	7.3±6.76	5.15±5.92	0.047
Absolute CD19⁺	180±281	84±147	0.003
% CD4⁺	45.1±10.28	33.38±11.88	<0.001*
Absolute CD4⁺	914±266	502±319	<0.001*
% CD4⁺CD45RA⁺	18.56±8.72	9.04±6.55	<0.001*
Absolute CD4⁺ CD45RA⁺	370±157	136±119	<0.001*
% CD4⁺CD45RO⁺	24.0±9.01	23.97±8.33	NS
Absolute CD4⁺ CD45RO⁺	532±303	361±219	0.025
% CD8⁺	30.66±13.77	42.52±15.51	0.010
Absolute CD8⁺	645±332	709±572	NS
CD4/CD8 ratio	1.94±1.27	0.94±0.53	0.003
CD4⁺CD45RA⁺/ CD4⁺ CD45RO⁺ ratio	0.94±0.73	0.40±0.32	<0.001*

**These p values were significant only after adjustment using Bonferroni´s test. NS: not significant.*

(376±135 and 355±94), which was even more pronounced in the MM and WM groups (199±185 and 203±165) (p<0.001). Differences in the CD4⁺CD45RO⁺ subpopulation were significantly less pronounced.

The CD4⁺CD45RA⁺/CD4⁺CD45RO⁺ ratio was significantly lower in patients with MM and WM than in subjects in the other groups (p=0.001), while the CD4/CD8 ratio remained similar.

When myeloma patients were separated on the basis of treatment status, differences persisted in percentage of CD4⁺ cells, percentage and absolute number of CD4⁺CD45RA⁺ cells, and in CD4⁺CD45RA⁺/CD4⁺CD45RO⁺ ratio (Table 1). The lowest values of these parameters were found in MM-OR, MM-PR, MM-TF/DP and WM patients (globally, those patients who had received some treatment), while they were higher in patients with MGUS, SMM and MM-D (untreated patients). We did not find changes in either CD4/CD8 ratio or in the CD4⁺CD45RO⁺ lymphocytes. Patients who had received some treatment showed a significant decrease in absolute lymphocyte numbers (p=0.004), without other cytopenias. The therapy-related alterations in lymphocyte subsets are shown in Table 2. Finally, as reported by other authors, we found no correlation between blood helper T-cell subsets and clinical stage of disease among patients with MM.

In summary, we consider, as did Kay *et al.*,[5] that therapy constitutes an important factor which needs to be addressed when lymphoid subpopulations are analyzed. Moreover, there may be some interesting implications from monitoring these subsets in the follow-up of patients with myeloma. As shown by Steingrimsdottir *et al.*,[6] the low number of CD4⁺ cells in patients after autologous stem cell transplantation is due to a persistently low level of CD4⁺CD45RA⁺ T-cells. The adult thymus contributes substantially to immune reconstitution (especially naive T-cells) in these patients,[7] and the CD4⁺ regeneration correlates quantitatively with the appearance of CD4⁺CD45RA⁺ T lymphocytes in the blood.[8] Future investigations must also take into account the possibility that CD45RA-RO⁺ cells could re-express CD45RA,[9] and the existence of new subsets within the CD4⁺CD45RO⁺ memory T-cells.[10] The approach to phenotyping of T-cell subsets is continuously changing and improving, and these new aspects may make the division of naive and memory cells more complex than initially thought.

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