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Expansion of melanoma-specific lymphocytes in alternate gamma chain cytokines: gene expression variances between T cells and T cell subsets exposed to IL-2 versus IL-7/15

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Abstract

We have previously demonstrated that expansion of activated tumor-sensitized T cells in interleukin (IL)-7/15 results in greater expansion and anti-tumor activity than expansion in IL-2. We sought to determine whether T cells exposed to IL-2 versus IL-7/15 exhibited distinct gene expression patterns. Lymphocytes were harvested from Pmel-1 mice immunized with B16-GMCSF melanoma cells, activated in vitro, and cultured in IL-2 or IL-7/15 for 1, 3 or 6 days. T cells were harvested and analyzed by microarray, real-time quantitative polymerase chain reaction (RT-QPCR), or sorted into T cell subsets and analyzed. We found significant differences in gene expression for T cells cultured in IL-2 vs. IL-7/15, starting at day 3. This was not a function of subset differentiation; when T cell were divided into subsets, the central memory (T_{CM}), effector memory (T_{EM}) and effector (T_E) T cells cultured in the IL-2 more closely resembled each other than the identical phenotypic subset exposed to IL-7/15. Thus, the differences in gene expression induced by culture in IL-2 versus IL-7/15 do not merely reflect differences in frequency of T_{CM} vs. T_{EM} vs. T_E cells, but rather reflect that the gene expression of those T cell subsets when exposed to different cytokines are fundamentally different.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Keywords

IL-2; IL-7; IL-15; T cell subsets; gene expression

INTRODUCTION

Adoptive immunotherapy has been shown to be an effective therapy in mice and human patients with melanoma (1–3). We have shown previously that when tumor-sensitized T cells from tumor-draining lymph nodes were activated with bryostatin 1 (B) and ionomycin (I) subsequent culture in the combination of IL-7 and IL-15 produced markedly increased expansion of cell numbers compared with IL-2, in both a melanoma and a breast cancer model (4–6). Anti-tumor efficacy of adoptive immunotherapy with cells expanded in IL-7/15 after activation with B/I was improved when compared with cells expanded in IL-2 (4,5). We also found that the alternate gamma chain cytokines favored preferential differentiation and expansion of CD8+ T cells toward a central memory (T_{CM}) phenotype, which some have suggested are more effective at inducing tumor regression than T effector (T_E) cells, which were more likely to develop with exposure to IL-2 (2,4,5). We sought here to determine whether there were differences in patterns of gene expression in T cells and T cell subsets exposed to IL-7/15 versus IL-2 that might further explain the dffierences in proliferation and *in vivo activity*.

MATERIALS AND METHODS

Mice

T cell receptor (TcR) transgenic Pmel-1 mice, with TcR specific for the peptide KVPRNQDWL derived from gp100 bound to class I of H-2b, were produced by breeding on-site from breeding pairs obtained from Jackson Laboratories (Bar Harbor, Maine). All guidelines of the Virginia Commonwealth University Institutional Animal Care and Use Committee, which conform to the American Association for Accreditation of Laboratory Animal Care and the US Department of Agriculture recommendations for the care and humane experimental use of animals, were followed.

Tumor cell lines

B16-GMCSF tumor cells were kindly provided by Dr. Richard Dutton (Trudeau Institute, Saranac Lake, NY). Melanoma cells were cultured in complete RPMI 1640 with 10% heat inactivated fetal calf serum, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 0.075% sodium bicarbonate, 2 mM L-glutamine, 100 U/ml penicillin, 100 g/ml streptomycin, 10 mM Hepes buffer, and 5×10^{-5} M 2-mercaptoethanol (Sigma, St Louis, MO). Tumor cells were harvested for inoculation of mice with 0.05% trypsin-EDTA (Invitrogen), washed twice with 1X PBS and resuspended in 1X PBS. Cells were filtered before injection through a 70um cell strainer. All cells were incubated in 250 ml T-flasks (PGC, Gaithersburg, MD) at 37°C in humidified air with 5% CO_2.

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Draining lymph node sensitization and activation

Pmel-1 mice were inoculated in the hind footpads with 1×10^6 B16-GMCSF cells. Ten days after footpad vaccination, popliteal tumor draining lymph nodes (DLN) were harvested under sterile conditions. DLNs were harvested and dispersed into single cell suspensions in complete RPMI media at 1×10^6 cells/ ml. The cells were then activated by incubation with 5 nM Bryostatin 1 (provided by Sigma Aldrich, St. Louis, MO) and 1 μM Ionomycin (Calbiochem, San Diego, CA) (B/I), and 80 U/ml of rIL-2 (Peprotech, Rocky Hill, NJ) at 37°C for 18 h. Cells were then washed three times with warm complete RPMI and resuspended at $1–2\times10^6$ cells/ml with either 40 U/ml of rIL- 2 or IL-7 + IL-15, each at 10 ng/ml. The cells were allowed to proliferate in culture for an additional 6 days and were split every 2–3 days in order to maintain $1x10^6$ cells/ml concentration. Additional cytokine at the above doses were also added when cells were split. Our previous work with this activation strategy has demonstrated that almost all remaining cells in culture after exposure to B/I and subsequently to cytokines are T cells.

Flow cytometry and sorting for T cell subsets

Cells isolated from DLNs and expanded as described above were stained with a panel of antibodies and analyzed or sorted based on surface marker expression on a BD FACS Aria™ II High-Speed Cell Sorter at days 0 (after B/I), 1, 3 and 6 of expansion. Fluorescent labeled Abs directed against the following markers were obtained from Biolegend: CD4 (GK1.5), CD8 (53–6.7), CD44 (IM7), CD62L (MEL-14). Appropriate isotype and single color controls were used in all cases. T cell subsets analyzed were T effector (T_E) CD44+CD62L-, T effector memory (T_{EM}) CD44+CD62Llow, and T central memory (T_{CM}) CD44+CD62Lhigh.

Microarray and real time quantitative polymerase chain reaction (RT-QPCR) preparation

Cultured lymphocytes were harvested at days 0, 1, 3 and 6 after activation, and without any fractionation, 1 million cells were suspended in 300 μL of trizol solution and then frozen for later analysis (“unsorted” cells). Microarray analysis was also performed on T cells that were sorted by FACS directly into 300 μL of trizol for each cell phenotype and then frozen for later analysis.

RNA Extraction

Total RNA was extracted and the quality evaluated using a sample processing method previously established in our laboratory(7). Total RNA was extracted from 1×10^6 cells (unsorted or sorted cells) using the MagMAX™-96 for Microarrays Total RNA Isolation Kit (InvitrogenTM Life Technologies, Carlsbad, CA) in an automated fashion using the magnetic particle processors MagMAX™ Express. RNA purity was judged by spectrophotometry at 260, 270, and 280 nm. RNA integrity as well as cDNA and cRNA synthesis products were assessed by running 1 μL of every sample in RNA 6000 Nano LabChips® on the 2100 Bioanalyzer (Agilent Technologies, Foster City, CA).

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Gene expression microarray analyses

The Affymetrix® protocol utilized for our microarray analyses has been previously described(7) and was used with the following modifications: Starting with 500 ng of total RNA, we performed a single-strand cDNA synthesis primed with a T7-(dT24) oligonucleotide. Second strand cDNA synthesis was performed with E.coli DNA Polymerase I, and biotinylation of the cRNA was achieved by *in vitro* transcription (IVT) reaction using the GeneChip® 3' IVT Express Kit (Affymetrix, Santa Clara, CA). After a 37°C-incubation for 16 hours, the labeled cRNA was purified using the cRNA cleanup reagents from the GeneChip® Sample Cleanup Module. As per the Affymetrix® protocol, 10 μg of fragmented cRNA were hybridized on the GeneChip® Mouse Genome 430A 2.0 array (Affymetrix Inc., Santa Clara, CA) for 16 hours at 60 rpm in a 45°C hybridization oven. The GeneChip® Mouse Genome 430A 2.0 array 230 provides a comprehensive coverage of the transcribed murine genome by including over 22,600 probe sets that analyze the expression level of over 14,000 well-characterized mouse transcripts. The arrays were washed and stained with streptavidin phycoerythrin (SAPE; Molecular Probes, Eugene, OR) in the Affymetrix® fluidics workstation. Every chip was scanned at a high resolution, on the Affymetrix® GeneChip Scanner 3000 7G according to the GeneChip® Expression Analysis Technical Manual procedures (Affymetrix). After scanning, the raw intensities for every probe were stored in electronic files (in .DAT and .CEL formats) by the GeneChip® Operating Software v1.4 (GCOS) (Affymetrix). Overall quality of each array was assessed by monitoring the 3′/5′ ratios for the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and the percentage of "Present" genes (%P). Arrays exhibiting GAPDH 3′/5′ < 3.0 and %P $> 40\%$ were considered good quality arrays.

RT-QPCR

RT-QPCR was used to validate gene expression levels of selected genes using TaqMan® chemistry. Probes and primer sets specific for detection of mouse RNA transcripts were purchased from Applied Biosystems, Foster City, CA. These included gene-specific probes for the following mouse genes: Igk-V28, assay ID# Mm01742005_g1; Ccr9, assay ID# Mm02620030_s1; Foxp3, assay ID# Mm00475162_m1; Lta, assay ID# Mm00440228_gH; Cdk6, assay ID# Mm01311342_m1; Jun, assay ID# Mm00495062_s1; Nov, assay ID# Mm00456855_m1. Gene-specific probes labeled with FAM (6-carboxyfluorescein) in the 5′ end, and with a dark quencher in the 3′ end were used for all the target genes of interest. For each sample, GAPDH was used as the endogenous control gene (assay ID# Mm99999915_g1) using a mouse-specific probe labeled with FAM (6-carboxyfluorescein) in the 5′ end, and with a dark quencher in the 3′ end. The experiments were performed in the ABI Prism 7500 Sequence Detection System (Applied Biosystems) using the TaqMan® Reverse Transcription and Universal PCR Master Mix Reagents. All the samples were tested in triplicate. The cycling conditions were 48°C for 30 min, 95°C for 10 min, 40 cycles of 95 °C for 15 s, and 60°C for 1 min. The 2– Ct method was used to calculate fold changes in the expression levels of the genes of interest(8).

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Statistical analysis

Microarray data analysis, background correction, normalization, and estimation of probe set expression summaries were performed using the log-scale robust multi-array analysis (RMA) method(9). Hierarchical cluster analyses were performed with the BRB-ArrayTools v3.1.0 (Biometric Research Branch, National Cancer Institute), an Excel add-in that collates microarray data with sample annotations. In order to identify differentially expressed genes between the different classes, we performed t-tests for each probe set from biological replicates in each class. Statistical significance for multivariate analysis to assess probe set specific false discovery rates (FDR) was performed by estimating the q-values, using the Bioconductor *q-value* package(10). Pearson's correlation coefficient was calculated to examine the relation between microarray and RT-QPCR results. $P < 0.05$ were considered significant.

Functional Analysis of Differentially Expressed Genes

A data set containing Affymetrix probe set IDs as gene identifiers and corresponding fold changes in expression levels and their associated significance (P-value) was uploaded into the Ingenuity Pathways Analysis (Ingenuity Systems, www.ingenuity.com) application. Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base (IPKB). These genes, called Focus Genes, were overlaid onto a global molecular network developed from information contained in the Ingenuity Pathways Knowledge Base. Networks of these Focus Genes were then algorithmically generated based on their connectivity, or interactions between one another. Biological networks were ranked by score, where the score corresponds to the likelihood of a set of genes being found in the networks due to random chance; that is, a score of 3 indicates that there is a 1/1,000 chance that the focus genes are in a network due to random chance. A score of 3 was used as the cutoff for identifying gene networks significantly involved in differences observed in IL-2 *versus* IL-7/15 treated cells.

RESULTS

Gene expression analysis for IL-2 and IL-7/15 expanded cells

To identify genes that were significantly differentially expressed, microarrays were performed in triplicate on unsorted T cells harvested after activation with B/I and expansion for 6 days in either IL-2 or IL-7/15. Sixty-three probe sets/genes had significantly different levels of expression between the two cytokine regimens (Table 1 – most significant 26 shown). In order to delineate the kinetics of the divergence in the gene expression patterns after B/I activation and separation of the cells into the two different cytokine culture conditions, we performed an unsupervised cluster analysis on T cells immediately after 18 hr. activation with B/I and then subsequently after 1, 3 and 6 days of expansion in either IL-2 or IL-7/15 (Figure 1). This analysis demonstrated that the two populations were similar after one day of exposure to the different cytokines, but gene expression patterns diverged dramatically based on their Euclidean distance on day 3 and remained different on day 6. To confirm the reproducibility of these observations, we performed a supervised cluster analysis on three biological replicates (Figure 2). The cluster analysis, based on 119 probe

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sets, demonstrated that the divergence after 6 days of exposure to either IL-2 or IL-7/15 was consistent.

RT-QPCR data

RT-QPCR was then performed to confirm the microarray results showing significant differences in gene expression between cells grown in IL-2 or IL 7/15. We chose 7 genes of interest that were among the most significantly different (higher or lower) in the microarray analysis. The seven genes chosen were Ccr9, Cdk6, Foxp3, Nov, Igk-V28, Jun and Lta. We observed the time course of differential gene expression at day 0 (after B/I pulse and prior to separation into different cytokine(s)), and after 1 (Day 1), 3 (Day 3) and 6 (Day 6) days of exposure to either IL-2 or IL-7/15. We then calculated the Pearson correlation coefficients (r) to determine whether the gene expression findings from the microarray data correlated with the PCR results and found significant positive correlations for the following genes: Ccr9 ($r = 0.975$; $p = 8.5\times10^{-6}$), Igk-V28 ($r = 0.982$; $p = 2.7\times10^{-6}$), Nov ($r = 0.845$; $p = 0.00413$), Foxp3 ($r = 0.842$; $p = 0.00433$), Lta ($r = 0.897$; $p = 0.00104$), and Jun ($r = 0.683$; $p = 0.04244$). The correlation for the Cdk6 gene was borderline positive but not significant ($r = 0.598$; $p = 0.08913$). Of those seven genes, Foxp3, Igk-V28 and Ccr9 demonstrated higher expression in T cells cultured in IL-2 for 6 days compared to T cells cultured in IL-7/15. The expression of Nov was higher in cells cultured in IL-7/15 for 6 days compared to those cultured in IL-2.

Functional Analysis of the Differentially Expressed Genes

The 63 differentially expressed probe sets/genes were overlaid onto a global molecular network developed from information contained in the IPKB. Networks of these genes were then algorithmically generated based on their connectivity, or interactions between one another. Five significant biological networks were ranked by score, identifying Cellular Growth and Proliferation, Hematological System Development and Function, and Inflammatory Response as the most relevant functional networks ($p = 10^{-41}$). The most significant network is shown in Figure 3.

Analysis of T cell subsets

One possible explanation for the differential gene expression patterns would be the differences in T cell differentiation we have observed when comparing cells cultured in IL-2 versus those cultured in IL-7–15. We have previously shown that IL-2 preferentially supports or expands T effector (T_E) populations while IL-7/15 preferentially expands T central memory (T_{CM}) populations in culture(4,5). In a repeat experiment using the same method of activation and expansion described above, this difference remained true (Figure 4). Moreover, it has been shown that different subsets of CD8+ T cells have different patterns of gene expression(11). In order to test the hypothesis that the differences in gene expression that we observed in different cytokines merely reflect different distributions of T cell subsets, we sorted T cells exposed to either IL-2 or IL-7/15 on day 3 and day 6 of culture into their CD8+ T cell subsets by flow cytometry- T central memory cells (T_{CM}), T effector memory cells (T_{EM}) and T effector cells (T_E) - using a FACSAria cell sorter. Interestingly, we found, after unsupervised cluster analysis, that differential phenotype distribution did not account for gene expression differences between different cytokine

conditions (Figure 5). In fact, the genes expressed in T_{CM}, T_{EM} and T_E cell subsets that were produced after expansion in either IL-2 or IL-7/15 for 6 days closely resembled the unsorted T cells stimulated with the same cytokines, rather than the subsets' gene expression patterns resembling each other, regardless of which cytokine they were exposed to. In other words, T_{CM}, T_{EM} or T_E cells produced after exposure to IL-2 had different patterns of gene expression from the corresponding T_{CM}, T_{EM} or T_E cell subsets produced after exposure to IL-7/15. Having made this observation, we then performed a supervised cluster analysis with the same samples, which confirmed the similar gene expression between unsorted T cells and T cell subsets T_{CM}, T_{EM} and T_E exposed to the same cytokine regimen for 6 days, but significant differences in gene expression between T_{CM} cells grown in IL-2 vs. IL-7/15 and likewise between T_{EM} cells and T_E cells grown in IL-2 versus IL-7/15 (Figure 6). We then used the same RT-QPCR probes as before to confirm the differential expression of genes among T cell subsets on day 3 and day 6 that were significant on the microarrays of unsorted cells. Thus, we found significant positive correlation for all the genes analyzed: Nov ($r = 0.989$; $p = 4.7\times10^{-5}$), Foxp3 ($r = 0.998$; $p = 4.2\times10^{-6}$), Igk-V28 ($r = 0.924$; $p = 1.5\times10^{-5}$), Lta ($r = 0.794$; $p = 4.8\times10^{-7}$), Cdk6 ($r = 0.712$; $p = 1.7\times10^{-5}$) and Ccr9 ($r = 0.561$; $p = 4.0\times10^{-6}$).

DISCUSSION

To date, few studies have been performed examining the gene expression profiles of T cell subsets(11,12). The consistent conclusion from these studies, however, has been that the gene expression patterns differ among T cell subsets, such that central memory, effector memory, effector and naïve T cells can be distinguished from each other based on their gene expression patterns. We had hypothesized, once we saw a distinct difference in gene expression between unsorted T cells cultured in IL-2 vs. IL-7/15, that this difference might be attributable to a greater frequency of T_E cells in the IL-2 group and a greater frequency of T_{CM} cells seen in the IL-7/15 group, as we have previously published(4–6). Surprisingly however, even when the T cells from these two cytokine exposure groups were sorted into their respective T_{CM}, T_{EM} and T_E subsets, these gene expression differences held true, differentiating T cells in the same subsets but grown in different cytokines from each other more than differentiating the subsets from each other. Thus, T_{CM} cells exposed to IL-2 in culture after activation with B/I are significantly different from T_{CM} cells exposed to IL-7/15 in culture after B/I activation, and so on for the other T cell subsets. The increased proliferation and survival of the cells expanded in IL-7/15 correlates with the findings from our functional pathway analysis, derived from differences in gene expression described here. Thus, among the 63 significantly altered probe sets/genes, we identified genes significantly involved in cellular growth and proliferation, hematological system development and function, and inflammatory response. For the seven genes chosen for RT-QPCR confirmation and analysis, four were markedly different between IL-2 expanded cells and IL-7/15 expanded cells both for unsorted as well as sorted T cells. The Nov gene (nephroblastoma overexpressed) was significantly more highly expressed in unsorted and all subsets of T cells exposed to IL-7/15. Nov has been shown to be involved in cell adhesion, migration, proliferation, differentiation, survival and angiogenesis through actions on integrin, NOTCH1 and fibulin 1c receptors(13–17). In cancer cells, it inhibits proliferation

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but promotes metastasis in patients with Ewing's sarcoma, melanoma and breast cancer(18–20). The other three genes, Ccr9, Foxp3 and Igk-V28 were more highly expressed in unsorted and sorted T cells exposed to IL-2 compared to T cells exposed to IL-7/15. Ccr9 has been shown to be important for migration of T cells(21), Igk-V28 gene's function is relatively unknown, and Foxp3 has been shown to have a strong correlation with CD4+ Treg cells(22). The importance of these genes in T cell subset function is unknown, and further studies are needed to elucidate why these differences exist between T cells cultured in IL-2 or IL-7/15. Most importantly, the "standard" way of evaluating T cell subsets used for immunotherapy based on phenotypic surface markers alone may not directly explain the relative efficacy of therapy with these cells *in vivo*. The differences in gene expression patterns we have found may well relate to increased expansion of cells in culture as well as the survival, trafficking or anti-tumor effectiveness of adoptively transferred T cells *in vivo*.

Acknowledgements

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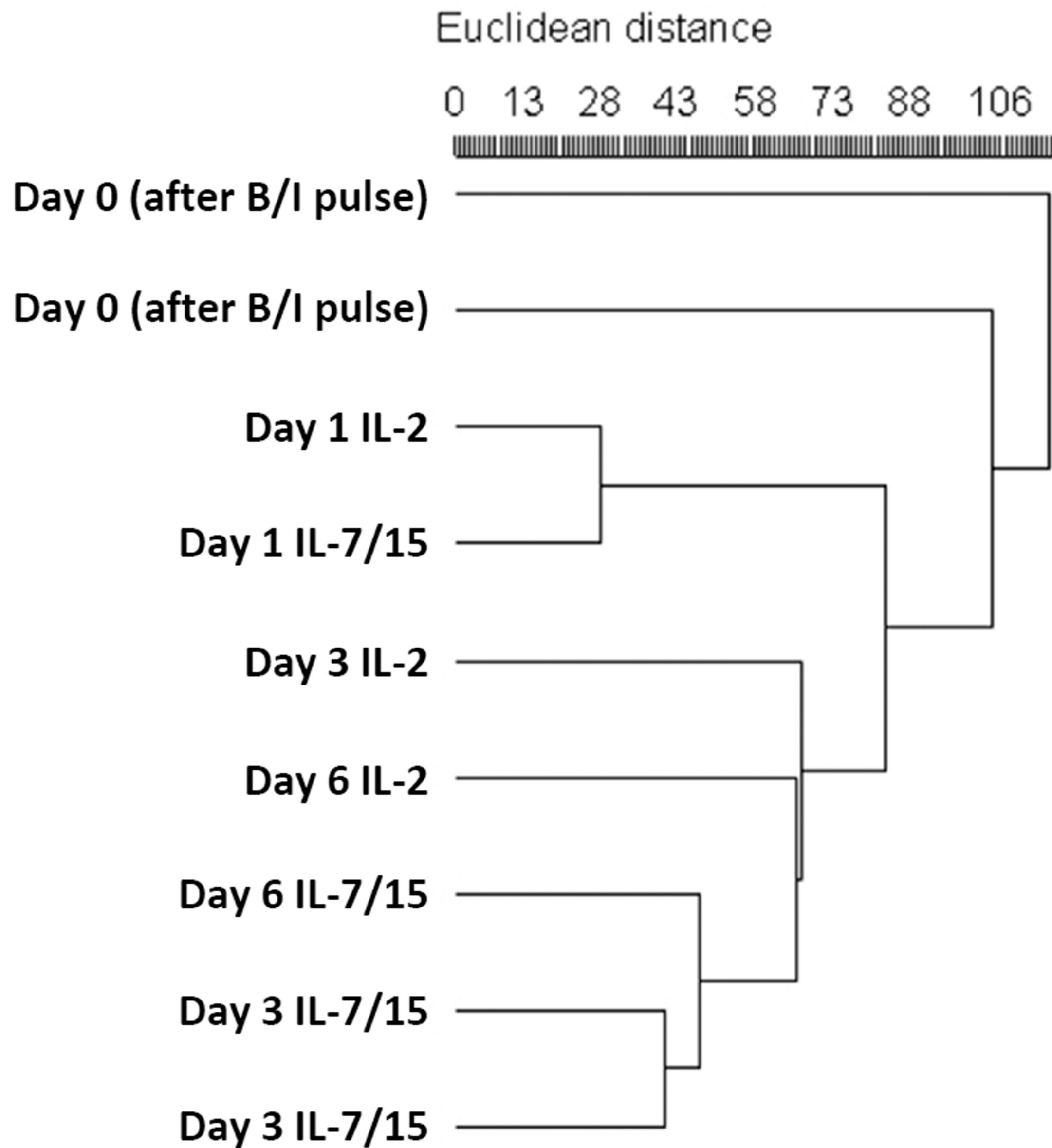

Figure 1.

Unsupervised cluster analysis performed on unsorted T cells analyzed at 4 time points: Day 0 – after activation with B/I and IL-2 for 18 hours and then washed, Day 1 – after 24 hours of exposure to IL-2 (40 U/mL) or IL-7/15 (each at 10 ng/mL), Day 3 – after 72 hours of exposure to IL-2 or IL-7/15 (re-dosed as above every other day), and Day 6 – after 144 hours of exposure to IL-2 or IL-7/15 (re-dosed as above every other day). Biological replicates were completed for Day 0 and Day 3 IL-7/15. B/I (bryostatin/ionomycin), IL (interleukin).

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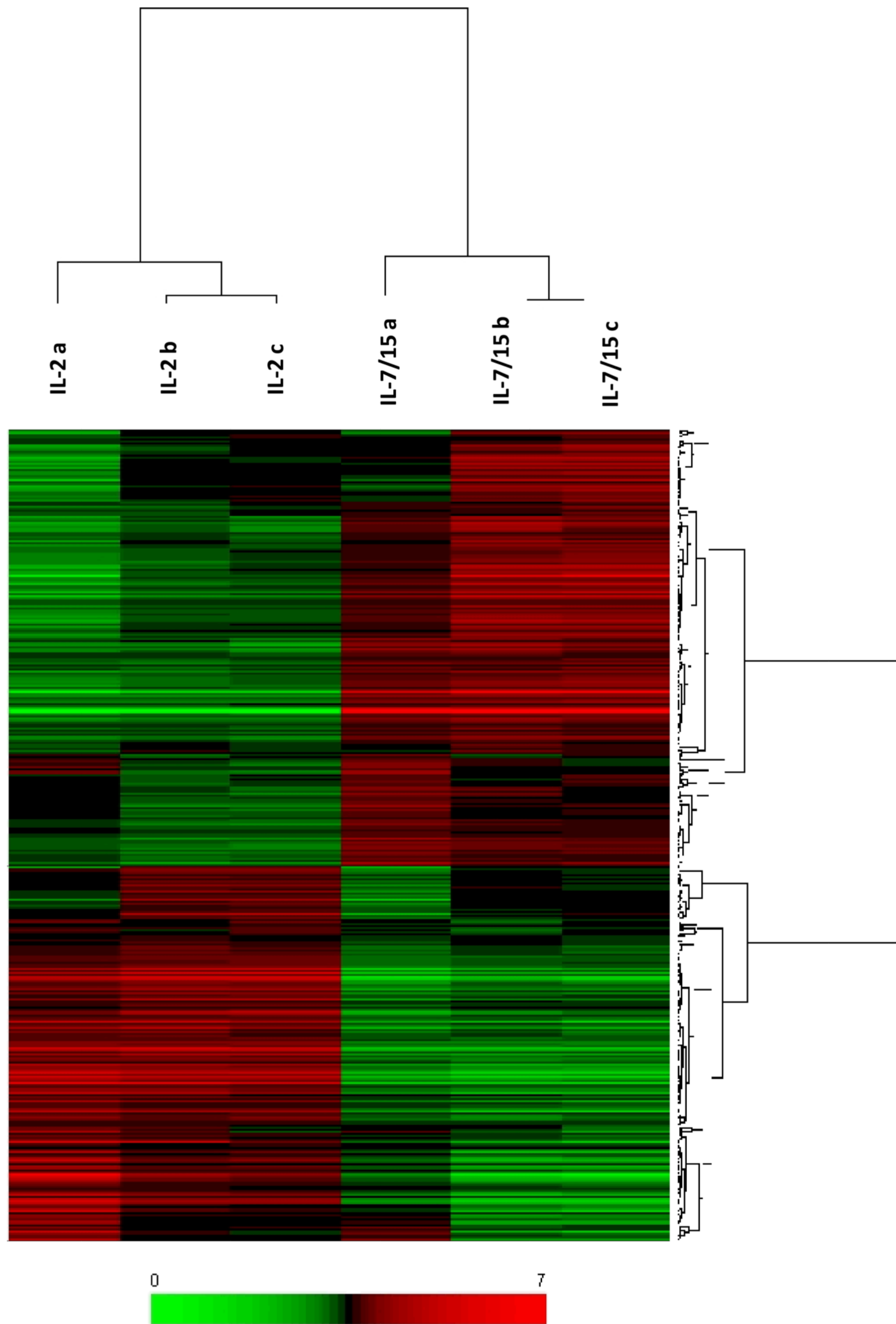

Figure 2.
Supervised cluster microarray analysis demonstrating that for 3 biological replicates (a, b and c) exposed to either IL-2 or IL-7/15 for 6 days, the gene expression remains consistent and different from each other. IL (interleukin).

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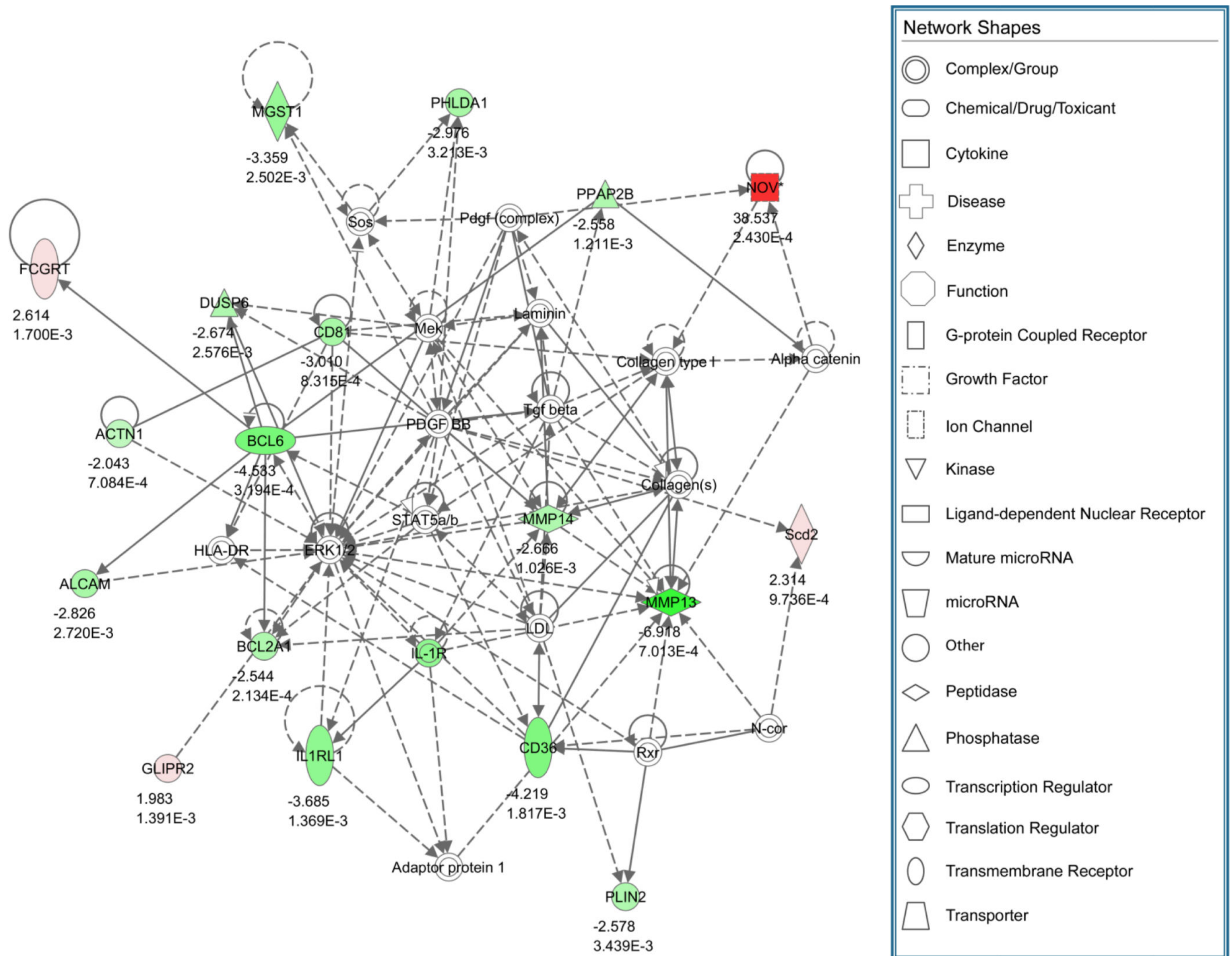

Figure 3.

Functional networks. Interconnection of significant functional networks, where gene nodes are shown in different shades of red, green, or white depending on being upregulated, downregulated, or no change, respectively, in T cells exposed to either IL-2 or IL-7/15 for 6 days. For each node, the fold change and significance are indicated. The most significant functional network corresponding to Cellular Growth and Proliferation, Hematological System Development and Function, and Inflammatory Response ($p < 10^{-41}$) is shown. The meaning of the network node shapes is also indicated.

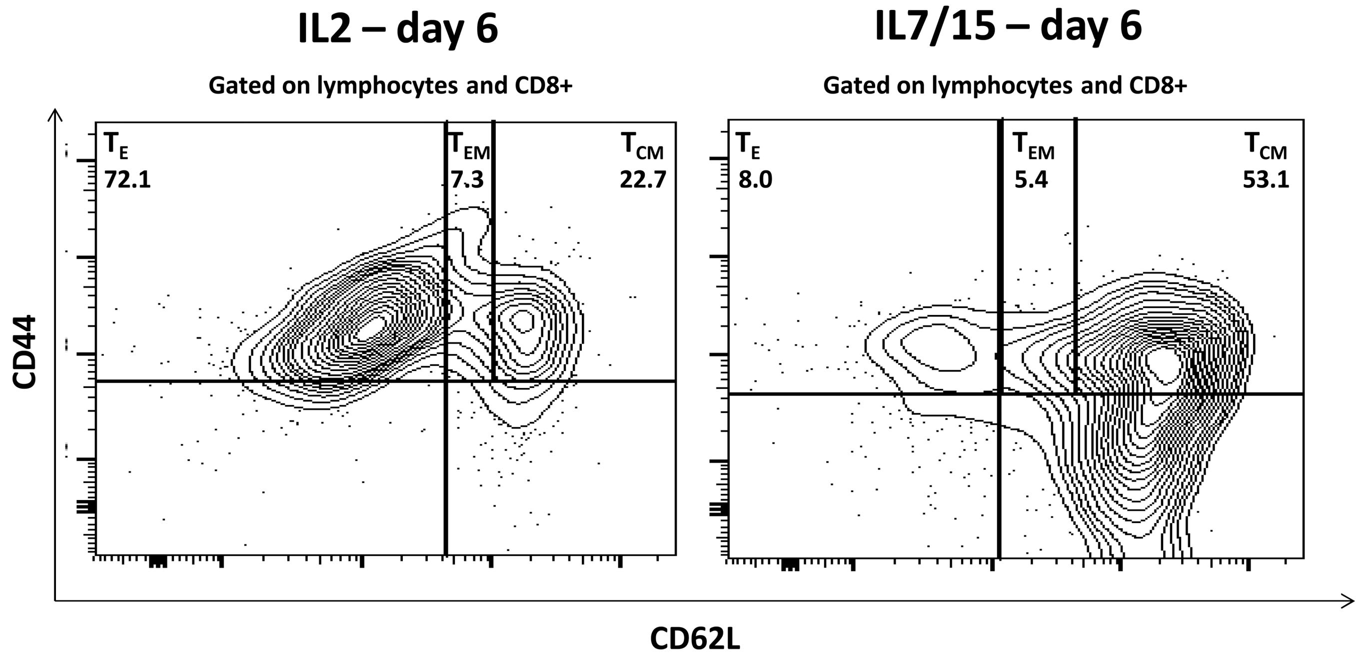

Figure 4.

Representative flow cytometry dot plot of T cells cultured for 6 days in IL-2 or IL-7/15 after activation with B/I. T cells exposed to IL-2 demonstrate a higher frequency of T effector cells whereas T cells exposed to IL-7/15 demonstrate a higher frequency of T central memory cells. IL (interleukin), T_E (T effector), T_{EM} (T effector memory), T_{CM} (T central memory).

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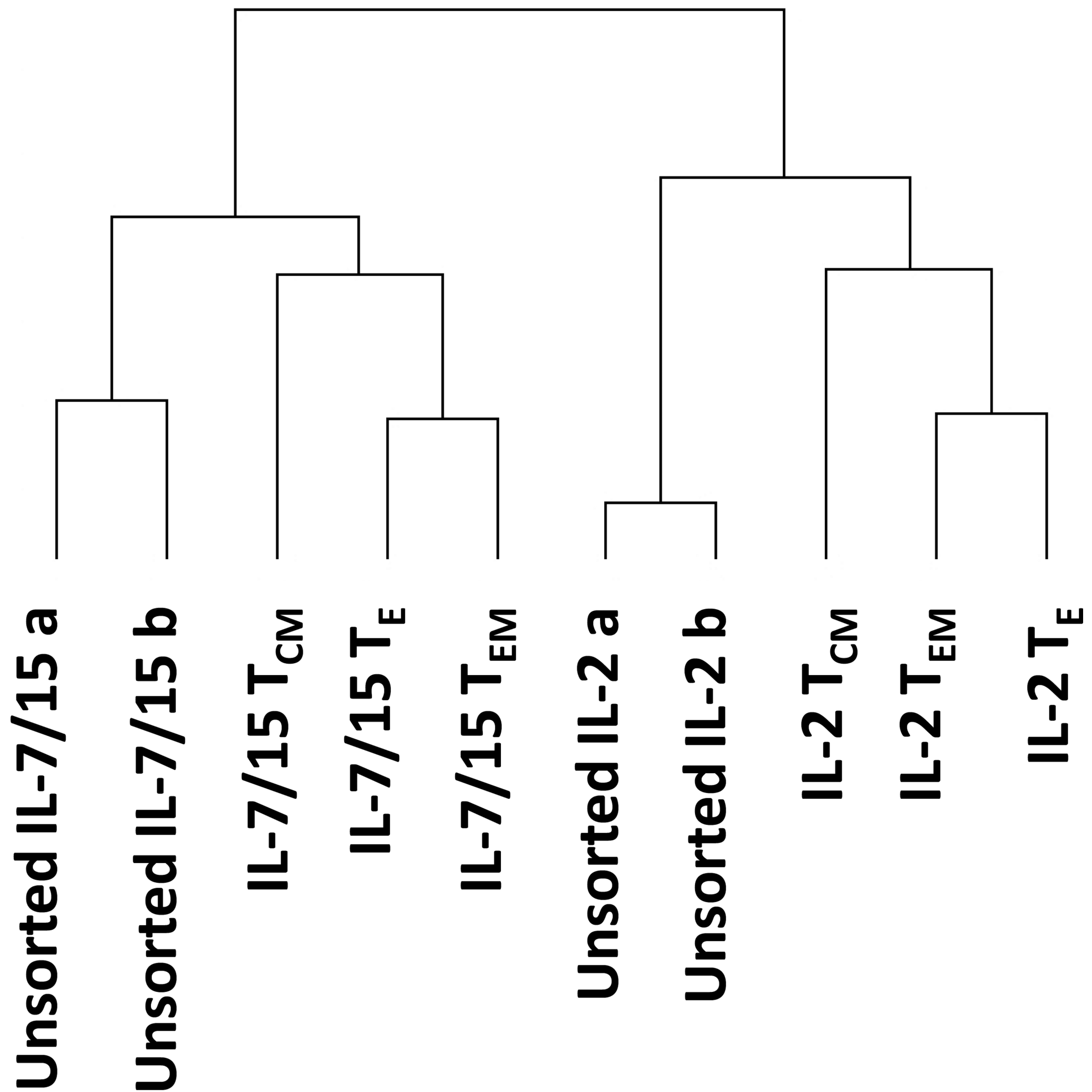

Figure 5.

Unsupervised cluster analysis of unsorted T cells after activation with B/I and 6 days of culture with IL-2 or IL-7/15 (two replicates: a and b) and T cells sorted into subsets: T_{CM}, T_{EM} and T_E after activation with B/I and 6 days in culture with IL-2 or IL-7/15. IL (interleukin), T_E (T effector), T_{EM} (T effector memory), T_{CM} (T central memory).

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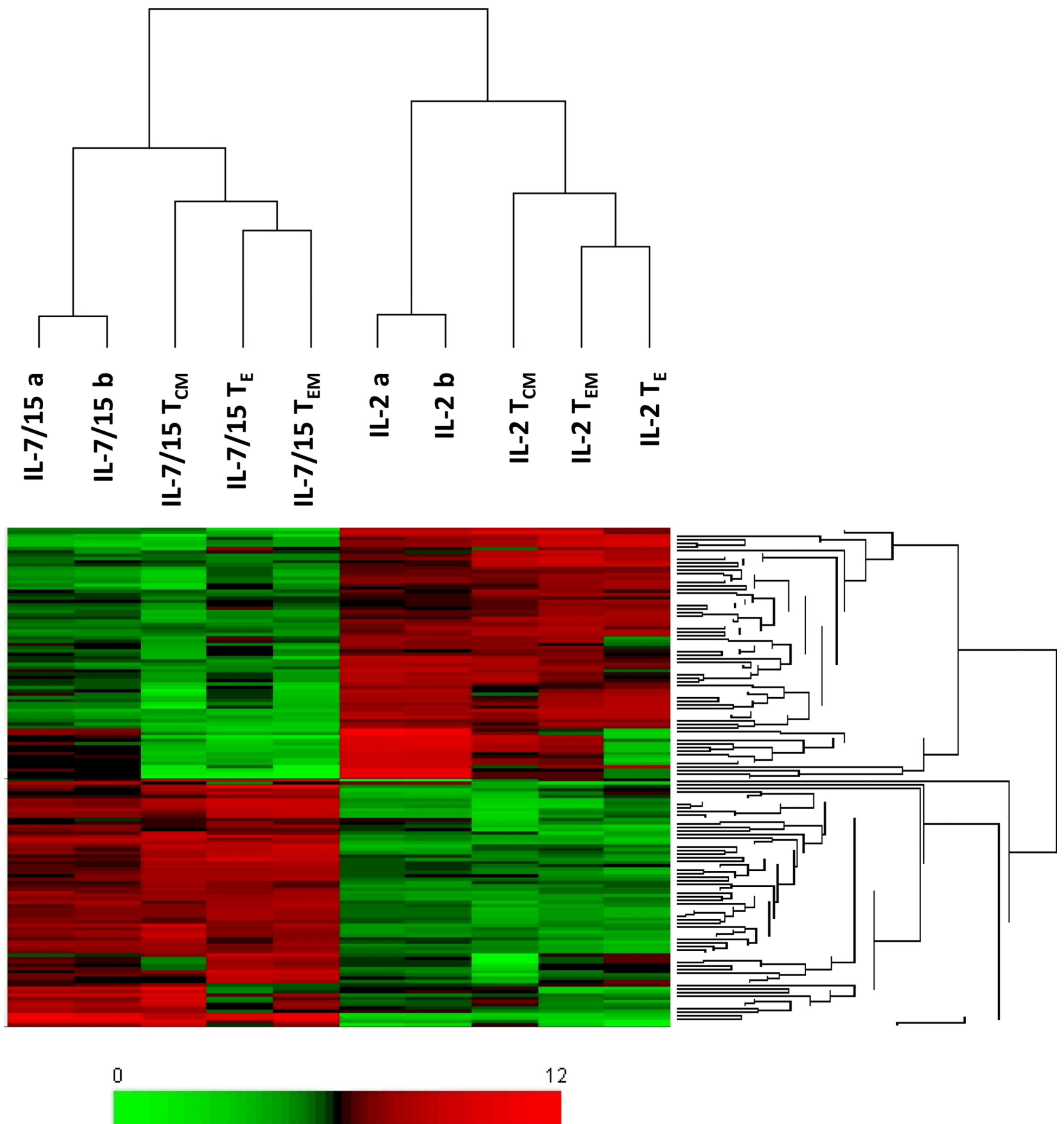

Figure 6.

Supervised cluster analysis of unsorted T cells after activation with B/I and 6 days of culture with IL-2 or IL-7/15 (two replicates: a and b) and T cells sorted into subsets: T_{CM}, T_{EM} and T_E after activation with B/I and 6 days in culture with IL-2 or IL-7/15. IL (interleukin), T_E (T effector), T_{EM} (T effector memory), T_{CM} (T central memory).

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Table 1

Twenty-six of 63 genes that were significantly differentially expressed between T cells cultured for 6 days in IL-2 vs. IL-7/15. These results are based on the analysis of three biological replicates for each cytokine. Seven of these were chosen for further analysis: Nov, Jun, Lta, Ccr9, Cdk6, Foxp3 and Igk-V28. IL (interleukin), FDR (false discovery rates).

Gene Symbol	IL-2 Mean (Log2)	IL-7/15 Mean (Log2)	Fold Change (Geometric)	p-value (α = 0.005)	q-value (FDR<15%)
Nov	2.91	8.18	38.5	2.43E-04	1.28E-01
Nov	4.12	8.81	25.8	8.06E-05	1.28E-01
Mylc2pl	6.53	10.23	13.1	1.41E-04	1.28E-01
Aldoc	7.19	9.56	5.2	2.91E-03	1.40E-01
Jun	6.35	8.45	4.3	2.09E-03	1.40E-01
Rcn1	7.85	9.49	3.1	6.15E-04	1.30E-01
Cdk6	4.71	6.29	3.0	2.10E-03	1.40E-01
Lta	8.44	9.99	2.9	7.83E-04	1.30E-01
LOC100044677 /// Tox	8.11	6.54	−3.0	3.91E-03	1.50E-01
Phlda1	8.99	7.42	−3.0	3.21E-03	1.42E-01
Cpa3	7.38	5.79	−3.0	2.69E-03	1.40E-01
Cd81	9.08	7.49	−3.0	8.31E-04	1.30E-01
Cnn3 /// LOC100047856	9.30	7.71	−3.0	1.65E-03	1.35E-01
Egr2	7.03	5.39	−3.1	3.09E-03	1.42E-01
Mgst1	5.50	3.75	−3.4	2.50E-03	1.40E-01
Il1rl1	10.22	8.34	−3.7	1.37E-03	1.33E-01
Lztfl1	9.47	7.57	−3.7	2.30E-03	1.40E-01
Ccl9	7.51	5.55	−3.9	3.19E-03	1.42E-01
Cd36	7.07	5.00	−4.2	1.82E-03	1.39E-01
Foxp3	6.93	4.83	−4.3	2.59E-03	1.40E-01
Bcl6	7.53	5.35	−4.5	3.19E-04	1.28E-01
Lpl	7.67	5.49	−4.5	1.07E-03	1.32E-01
Rgs2	8.58	6.34	−4.7	4.08E-03	1.50E-01
Rgs2	8.35	5.84	−5.7	2.68E-03	1.40E-01
Igk-V28	9.22	6.65	−6.0	2.06E-03	1.40E-01

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Gene Symbol	IL-2 Mean (Log2)	IL-7/15 Mean (Log2)	Fold Change (Geometric)	p-value (α = 0.005)	q-value (FDR<15%)
2010205A11Rik	11.12	8.46	−6.4	4.35E-04	1.28E-01
Igk-V28	11.21	8.54	−6.4	1.94E-05	9.55E-02
Mmp13	6.54	3.74	−6.9	7.01E-04	1.30E-01
Ccr9	8.06	5.18	−7.4	4.23E-04	1.28E-01
Igk-V28	10.71	7.74	−7.8	5.60E-04	1.30E-01