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Kinetics of lymphocyte activation and senescence markers in chronic HCV-infected patients on PEGIFNα-2a/Ribavirin/Telaprevir

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ABSTRACT

Activation and Senescence Markers in HCV-infected Patients on PegIFN/RBV/Telaprevir

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BACKGROUND: Immune activation and senescence described in chronic viral infections is associated with an increase in morbidity and mortality. No data were reported in HCV-infected patients (pts). We describe the immunological markers of activation, senescence and exhaustion in pts initiating PegIFN/RBV/Telaprevir tritherapy.

METHODOLOGY: Pts with a prior PegIFN/RBV failure who initiated tritherapy (D0) and obtained SVR24 were included. CD4 and CD8 activation (DR+), maturation (naive, memory T cells), senescence (CD57+CD28-), regulatory T cells, and PD1+T cells were measured at D0, W12, W48 and W52-72. Kinetics was described during the 3 periods: D0-W12 tritherapy; W12-W48 bitherapy; W48-W72 off treatment. D0 data were compared with 20 healthy donors (HD).

RESULTS: 83 pts were included: 66% males, median age=57 years, infection duration=30 years, HCVRNA=1643000 IU/mL, fibroscan =10 kPa (36% F4).

There is no significant differences for CD4+ and CD8+ senescence or maturation between pts at D0 and HD. CD4+ and CD8+ activation was higher in patients (p=10-3 and 0.07) than in HD, although within the normal range.

D0-W12 period: significant decrease in senescent (*P* =10-4) and memory cells (*P*=10-2 to 10-4) without variation in activated CD4+ and CD8+; increase in Treg (*P*=10-3) without variation in % PD-1+CD4+ or CD8+.

W12-W48 period: No variation occurred.

W48-W72 period: increase in memory (*P*<10-4 to *P*<10-2), senescent (*P*=10-2) and activated (*P*<10-4) CD4+ and CD8+, while naive T cells decreased (*P*<10-4). **CONCLUSIONS:** Chronic HCV replication for more than 30 years is associated neither with a CD8+ clonal expansion, nor with lymphocyte activation or senescent status in the peripheral blood. No association was found between activation and fibrosis. We speculated that the immunological variables kinetics on successful IFN-based therapy may be due to variations in lymphocytes homing: decrease in memory and senescent lymphocyte subsets on suppressive tritherapy, increase in memory, senescent and activated lymphocytes after the end of IFN exposure without detectable HCV. The intrahepatic immune response does exist and may be under the regulation of lymphocytes and Treg homeostasis. It seems relevant to follow the persistence of this activated status at the end of IFN exposure and to evaluate its clinical impact, notably in terms of fibrosis evolution and comorbidities. This observed immune paradox may be of interest for the deciphering of new (IFN-free) therapeutic strategies.

BACKGROUND

Despite effective antiretroviral therapy, chronic HIV- or HIV-HCV co-infections can trigger ongoing immune activation and inflammation that was more pronounced than in the mono-infected HCV patients.

The suppression of HCV replication by effective PEGIFNα/RBV therapy in HIV-HCV co-infected patients reduced immune activation (CD8+CD38+) (*Gonzalez et al. J Virol 2009*) as well as inflammation markers and endothelial dysfunction (*Guzman et al. JAC 2011*). Others immune activation markers like sCD14 and CD163 were correlated with the severity of liver disease.

This immune activation may contribute to the increase of morbidity and mortality. Our aim was to describe the immunological markers of activation, exhaustion and senescence in a population of chronic HCV mono-infected patients initiating a PEGIFNα/RBV/Telaprevir therapy and their link with the severity of fibrosis.

OBJECTIVE

To describe kinetics of immunological markers according to three time frames in patients with Sustained Virological Response (SVR24):

1- Triple therapy D0-W12 under PEGIFNα-2a/RBV/Telaprevir
2- Bitherapy W12-W48 under PEGIFNα-2a/RBV
3- After treatment stop W48-72

PATIENTS AND METHODS

Patients with a prior PEGIFNα-2A/RBV non response who had initiated (D0) a successful PEGIFNα-2a/RBV/Telaprevir (SVR24: undetectable HCV-RNA at W72) at the Bordeaux University Hospital Hepato-gastroEnterology unit were included.

The below described immunological markers were analyzed at D0, W4, W8, W12, W24, W48, W52, W60, W72.

- Immune Activation: CD3+DR+CD4+ and CD3+DR+CD8+
- Senescence: CD3+CD4+CD57+28- et CD3+CD8+CD57+28-
- Lymphocyte Maturation: naives CD4+, CD8+ (TN:CD45RA+CD27+), effectors memory (TEM:CD45RA-CD27-); central memory (TCM:CD45RA-27+); terminal differentiation (TEMRA:CD45RA+27-).
- Suppressive subsets:
  - Regulatory T cells
  - PD1+T cells

The Mann–Whitney U test was used to compare Immunological parameters between patients (D0) and healthy controls. The Kruskal-Wallis test was used to test for differences in immunological parameters according to fibrosis severity stages. Evolution of the above listed immunological markers were analyzed by a piecewise linear mixed model with three slopes (D0 – W12; W12-W48; W48-W72). The correlation between individual baseline value(s) and the subsequent slope(s) was handled through an unstructured covariance matrix of random effects.

RESULTS (1)

66 patients were included and are described for baseline characteristics in Table 1. Immunological markers found at baseline and during follow up are presented in Tables 2 and 3 and evolution of immunologcial markers are also presented in figures 1 to 10.

Table 1: Description of the patients population at D0

Patients Characteristics, n=66	n	%
Gender, male	43	(65.2)
Age at D0 start of therapy (years)	57	(48; 66)
Years since HCV infection diagnosis	30	(25; 32)
Contamination mode		
transfusion/surgency	32	(48.5)
Drug users	18	(27.3)
Others	15	(22.7)
IL28 rs8099917		
GG	6	(9.1)
GT	20	(30.3)
TT	33	(50)
Fibroscan value, kPa	10	(6; 15)
Fibrotest value	1	(0.6; 0.9)
Score fibrose		
F0 - F1	20	(30.3)
F2	10	(15.2)
F3	18	(27.3)
F4	30	(36.1)
HCV viral load, logUI/ml, median (IQR)	6.2	(5.8; 6.4)
HCV genotype		
1	9	13.6
1a	23	34.8
1b	33	50

Table 2: Comparison of immunological markers between healthy donors (HD, n=20) and D0 patients

Parameters, médian (IQR)	Healthy Donors (n=20)		D0 patients (n=23)		HD vs D0 patients, *P*
CD4 VA	696	(583; 821)	747	(540; 983)	0.46
CD4 (%)	43.2	(37.7; 48.9)	49.7	(41.7; 53.3)	0.16
TN among CD4, %	43.3	(32.0; 55.8)	41.8	(16.7; 48)	0.40
TEMRA among CD4, %	0.8	(0.2; 3.9)	0.42	(0.2; 4.2)	0.82
HLA-DR+ among CD4, %	5.6	(3.8; 6.4)	7.1	(5.6; 9.3)	**0.01**
CD57+CD28- among CD4, %	0.7	(0.1; 4.0)	0.38	(0.03; 4.3)	0.80
CD8 VA	326	(269; 444)	329	(239; 544)	0.57
CD8 (%)	20.4	(17.9; 25.7)	21.7	(17.5; 27.5)	0.52
TN among CD8, %	48.4	(39.6; 56.5)	45.2	(34.1; 66.8)	0.46
TEMRA among CD8, %	14.2	(8.2; 34.7)	17.8	(4.5; 31.9)	0.68
HLA-DR+ among CD8, %	10.5	(6.5; 18.4)	12.5	(8.1; 24.4)	0.26
CD57+CD28- among CD8, %	15.3	(9.2; 26.6)	15.1	(3.6; 32.5)	0.82
CD4/CD8 ratio	2.2	(1.5; 2.8)	2.1	(1.6; 2.8)	0.87

Immunological markers at D0 and comparison with Healthy Donors

In HCV infected patients (time since diagnosis: median 30 years) with a viral load of 6.2 log IU/ml:

- CD4+DR+ were higher in patients HCV compared to HD (P=0.01)
- No significant différence for both CD4+ and CD8+ T lymphocyte senescence, maturation, CD4/CD8 ratio (usually inverted in viral infection) between patients and HD.

Immunological markers at D0 and fibrosis severity

There were no statistical significant association between CD4+ and CD8+ T activation/senescence and fibrosis severity.

RESULTS (2)

Table 3: Description of immunological markers: Data for 23 patients (D0) , 27 (W2), 38 (W4), 19 (W8), 38 (W12), 32 (W24), 21 (W24), 33 (W52), 31 (W60) and 40 (W72). In Table 3 we present data for the following time points: D0 (start of PEGIFNα-2a/RBV/Telaprevir triple therapy), W12 (end of triple therapy), W24, W48 (end of PEGIFNα-2a/RBV bitherapy), W72 (SVR24).

	D0	W12	W24	W48	W72
CD3					
VA, mm3	1172	443	491	540	994
%	75.5	79.4	75.5	68.9	69.9
CD4					
VA, mm3	747	308	342	378	586
%	49.7	55.1	50.3	47.1	43.3
HLA-DR, %	7.1	6.3	5.7	6.9	11.3
CD57+CD28-, %	0.4	0.2	0.6	0.1	0.2
TN, %	41.8	45.5	35.7	39	35.9
TCM, %	48.6	49.7	52.3	54.4	54.1
TEM, %	8.1	5.8	5.2	6	7.4
TEMRA, %	0.4	0.3	0.8	0.3	0.6
Treg, %	2.7	3.8	3.6	3.6	3.7
PD1+, %	23.5	23.9	24.6	27.1	24
CD8					
VA, mm3	329	104	121	123	278
%	21.7	17.5	16.8	16.1	21.5
HLA-DR, %	12.6	12.9	10.2	9	26.4
CD57+CD28-, %	15.1	9.1	11.8	5.9	17.7
TN, %	45.3	61.3	58.1	69.1	47.7
TCM, %	28	21.3	19	15.8	26.4
TEM, %	5.3	4	3.7	2.8	5
TEMRA, %	17.8	7.5	11.1	7.2	13.4
PD1+, %	24.1	24.8	23.3	24.8	25.6
CD4/CD8 ratio	2.1	3.2	2.9	3	2

Immunological markers evolution between D0 and W12 under effective tritherapy:

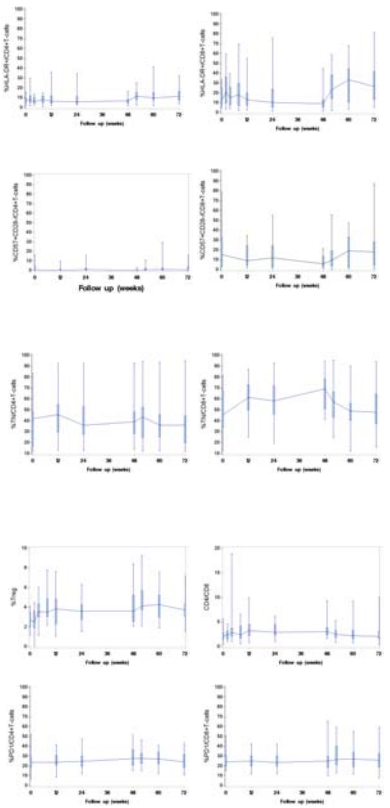
- We observed a statistically significant decrease of absolute count of total lymphocytes, CD4+ and CD8+ T cells ($P<10^{-4}$).
- The decrease in CD8- T-cells was more pronounced, resulting in increased percentages of CD4-T-cells and of CD4/CD8 ratio (P=0.04).
- There was no signficant evolution of activated CD4+ and CD8+ T subsets despite the suppression of viral replication (P=0.94 and P=0.41, respectively).
- Senescent CD4+ and CD8+ T subsets decreased by an estimated mean of -1.08% (P=0.07) and -5.56% (P = 0.01), respectively.
- Naive CD4 T and CD8 T cell subsets increased significantly by an estimated mean of +6% (P<10-4) and +11.6% (P<10-3).
- Suppressive lymphocyte populations: increase in regulatory T cells percentages (estimated mean of +0.7%, P=0.002), with no variation in CD4+PD-1+ or PD-1+CD8+ percentages (P=0.22 and P=0.06, repsectively)

Immunological markers evolution between W12 and W48 under effective PEGIFN/RBV bitherapy :

- Moderate increase in activated CD4+ and CD8+ T subsets (estimated mean +1.8%, P=0.01 and estimated mean +4.32%, P=0.05, respectively).
- No significant changes senescent CD4+ and CD8+ T subsets (P=0.18 and = 0.48, respectively).
- Naive CD4 T decreased significantly (-3.4%, P<10-3) and naive CD8 T cells also showed a decrease without being statistically significant (-1.44%, P=0.32).
- Significant increase in regulatory T cells percentages (estimated mean +0.6%, P=0.005) and in CD4+PD-1+ or PD-1+CD8+ percentages (estimated mean +3.6%, $P<10^{-4}$ and +3.96%,P=0.004, respectively).

Immunological markers evolution between W48 and W72 (W24 after the cessation of PEGIFN/RBV treatment):

- There was an increase of absolute CD4+ and CD8+ T lymphocytes cell count ($P<10^{-4}$).
- Normalization of the CD4/CD8 ratio close to D0 values.
- We observed a significant increase of activated CD4+ and CD8+ LT subsets: estimated mean +3.4% for DR+CD4 T cells (P=0.004) and +11.52% for DR+CD8 T cells ($P<10^{-3}$).
- Senescent CD4+ and CD8+ LT increased significantly by estimated mean +2.64% ($P<10^{-3}$) and +10.32 ($P<10^{-4}$).
- Naive CD4 T cells continued to decrease (-2.3, P=0.06) and naive CD8 T cells decreased significantly (-8.9%, P<10-4).
- Suppressive lymphocyte populations: no significant variation in Treg or CD8+ PD-1+ percentages but a significant decrease in CD4+PD-1+ percentages (estimated mean -4.32%, $P<10^{-4}$).



DISCUSSION- CONCLUSIONS

In the peripheral blood, chronic HCV replication (>6 logIU/ml) for more than 30 years is associated with :

- A moderate increase in immune activation (CD4+DR+) compared to HD, although within the normal range, without association with fibrosis severity.
- No expansion of senescent or memory T lymphocytes compared to HD.
- No CD8+ lymphocytes clonal expansion, as classically described in chronic viral infection with the occurrence of comorbidities.

The effective PEGIFN/RBV/TELAPREVIR triple therapy with HCV replication suppression is associated with:

- Moderate increase in % of activated T lymphocytes
- An increase in suppressive lymphocyte subsets

Between the cessation of treatment and the SVR24, we observed:

- An increase in immune activation (CD4+DR+ and CD8+DR+) to a level higher than D0 level
- An increase in memory and senescents CD8+ T lymphocytes
- A decrease in naive T cells

Our data suggest a similar modulation in peripheral blood of CD4+ and CD8+ T-cell subsets by PEG-IFN2a/RBV/Telaprevir in vivo in SVR24 patients.

Contrary to the observations in HIV-HCV co-infected patients on bitherapy (Massanella 2010), changes did not return to baseline levels after treatment cessation with the gain of activated and senescent T cells, and the loss of naive CD4+ T-cells.

These data are in contrast with the described reduction in T-cells activation (measured by CD38 expression) induced by PEG-IFN treatment in HIV-HCV co-infected patients [Gonzalez VD, J Virol 2009].

After an initial increase under triple therapy, bitherapy with PEGIFN2a/RBV reduced the percentage of naive CD4+T-cells. This reduction remained significant at week 72. A similar finding has been previously reported and related to a reduced thymic output under IFN-based therapy. Our data suggest that damage of thymic function after PEG-IFN2a-based therapy should be evaluated.

Treg frequencies increase during IFNa-based therapy and remained above baseline after cessation of therapy in patients who became negative for HCV RNA, as described for this subset in the liver [Claassen MA, J Virol 2011].

Little information exists on the mechanisms and the relevance of these changes.

✓Circulation of T cells and homing in the peripheral blood seems to be altered by IFN (endo- or exogenous) and/or virus.

✓The major increase in activated and senescent T lymphocytes after the cessation of IFN-treatment raises the question of the liberation of these cells in the peripheral blood after their trapping in the liver compartment during viral replication period or under IFN treatment.

✓ It seems relevant to follow the persistence of this activated status and to evaluate its clinical impact, notably in terms of fibrosis evolution and comorbidities.

✓This observed immune paradox may be of interest for the deciphering of new IFN-free therapeutic strategies.

✓The understanding of exogenous/ endogenous inflammation mechanisms and of immune activation, not only on T lymphocyte cells, but also on other immune cell partners (monocytes, NK, polynuclear) is needed before prescription of new therapeutic options (induction of inhibitory molecules like PD-1, CTLA-4, Tim-3 on T lymphocytes) that could be considered to reduce the progression of inflammatory liver disease.

✓The study of the association of activation/senescence profiles and suppressive cells at the end of therapy with the fibrosis evolution is planned.

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