INSTITUT COCHIN Immunologie hématologie

Rapport Hcéres

agence d'évaluation de la recherche
et de l'enseignement supérieur

Section des Unités de recherche

Evaluation report

Research unit :

Department of Immunology and Hematology

of the Cochin Institute

University Paris 5

March 2009

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et de l'enseignement supérieur

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Of the Cochin Institute

University Paris 5

Le Président de l'AERES	Section des unités de recherche
	Le Directeur
Jean-François Dhainaut	**Pierre Glorieux**

mars 2009

Evaluation report

The research unit :

Name of the research unit : Department of Immunology and Hematology

Requested label : UMR CNRS, UMR_S INSERM

N° in case of renewal :

Head of the research unit : Mr Pierre Olivier COURAUD

Head of the Immunology Department: Mr Alain TRAUTMAN

University or school :

University Paris 5

Other institutions and research organization:

INSERM

CNRS

Dates of the visit :

December 8-10, 2008

Members of the visiting committee

Chairman of the commitee :

Mr Roland LIBLAU, CPTP, Toulouse

Other committee members :

Mr Doreen CANTRELL, Dundee, UK

Mr Salvatore VALITUTTI, CPTP, Toulouse

Ms Kathleen FRESON, University of Leuven, Belgique

Mr Christian JORGENSEN, INM, Montpellier

CNU, CoNRS, CSS INSERM, INRA, INRIA, IRD... representatives :

Ms Katrin TARTE, Rennes, INSERM CSS representative

Mr Joël PESTEL, Lille, CNRS representative

Mr Bruno QUESNEL, Lille, CNU representative

Observers

AERES scientific representative:

Mr Marc BONNEVILLE

University or school representative:

Mr Bruno VARET, Université Paris 5

Ms Marie-Christine LABASTIE, Université Paris 5

Research organization representatives :

Ms Christine TUFFEREAU, INSERM representative

Ms Evelyne JOUVIN-MARCHE, CNRS representative

Specific appreciation team by team

Team 28 : Regulation of alternate NF-KB pathway

This young team is headed by a senior researcher with a CR1 INSERM position and includes also 2 post-doc, 1 PhD student and a research assistant (IE CNRS). Another senior researcher will soon join the team.

The team leader has initially identified an alternative NF-KB signalling pathway during her post-doctoral training and has developed an original and well-funded research project focused on 2 main goals: 1) to identify new RelB regulators and interacting partners using a molecular strategy involving the *in vivo* biotinylation of selected templates followed by protein purification and analysis; 2) to characterize the role of RelB in the control of cell proliferation and apoptosis in B cells *versus* non lymphoid cells, in particular owing to the recent and original finding that RelB modulates p53 protein expression and activity and could slow down solid tumor development. In addition, the new researcher will develop a new project aiming at targeting RelB pathway in B-cell lymphoma and CLL. The latter research field is very competitive and this line of research less well described than the two other axes. Finally, the team is involved in a translational collaborative network that has recently applied to the Medicen Paris Region, Pôle de Compétitivité and aims to develop new and specific NF-KB inhibitors.

Strengths :

- Young and dynamic team with very good national and international collaborations that compensate for the small size of the group.
- Very focused work on the NF-KB pathway for which they have international visibility and obtained good funding.
- Innovative projects supported by interesting tools and skills.
- Strong and convincing recent data on the crosstalk between p53 and RelB and on the identification of new RelB modulators.
- Connection with industrial partners.

Weakness:

The gap in publications could hopefully be filled soon by the new data that are currently submitted for publication.

Recommendations:

This promising group should focus its main investment on the characterization of p53/RelB interaction through the identification of specific clinical targets that would allow them to convincingly demonstrate the biological relevance of their intriguing findings.

Moreover, given the high level of international competition in this field, the originality and strength of the leukemia/lymphoma project should be more detailed in relation with normal B-cell biology.

Nom de l'équipe : Regulation of alternate NF-KB pathway

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
B	B	B	NN	A

Team 29 : Physiopathology of melanoma, combined chemo- and immuno-therapy

The previous team has been largely reorganised with the departure of one senior scientist in May 2006 and the recent arrival of a CR1 from Institut Gustave Roussy and a research engineer previously working in a private company. The IGR researcher is a senior scientist recognized for her expertise on the regulation of NK and T cells by KIR receptors and will reinforce the project on human melanoma. The research engineer is a junior scientist who managed projects on anti-tumor vaccines at IDM Pharma for 8 years. She has a great experience of on T-cell tolerance and anti-tumor immunity and consequently could have a dynamic effect for the group. Finally, the reshaping of the team opens new opportunities to develop original studies on T and NK cell reactivity in human tumors.

This team has focused its main research program on the mechanisms of immune suppression in the tumor microenvironment. Parallel investigations are carried out both in a mouse model and in human. Based on a original mouse model of spontaneous metastatic melanoma (RET mice), they reported that the development of vitiligo is associated with control of tumor growth through a CD8+ T cell dependent mechanisms. Interestingly in the RET melanoma model, tumor-associated macrophages (TAM) have a key influence on tumor progression. Indeed, as suggested by a transcriptome analysis, macrophages were described as playing a beneficial or detrimental effect depending on their polarization status, as previously observed for T cells or dendritic cells. Other projects have been initiated to precise the regulatory function of NK lymphocytes and tumor-infiltrating T cells. The potential involvement of the inhibitory receptor PD-1 and innate immune response components are investigated. Finally, in close collaboration with clinicians, new interesting experimental approaches (microarrays) were developed to elucidate the effect of chemotherapy on the local anti-tumor immune response in melanoma patients.

The project will consist in elucidating the role of myeloid cells during spontaneous development of melanoma. Among the main items, the team will pay attention: 1) to the role of macrophages in tumor immune escape, by using mouse model and analyzing the TAM signature at various stages of tumor development and in response to chemotherapy in human biopsies; 2) to study the function of TILs and NK cells in contact with melanoma cells in human biopsies (role of PD-1 and of the phosphatase SHP-1); 3) to the role of KIR (inhibitory NK receptors of Ig type) in the regulation of T-cell functions using the mutant cell lines

Strengths

- The team is the result of new organization and represents a nice mixture of clinicians and more basic researchers.
- All personal competences put together might help to elucidate the regulatory role of macrophages in the development of tumors.
- The team has a very faithful model of spontaneous melanoma that will be very useful for investigating the role of immune cells infiltrating tumors, mainly macrophages.
- Good publication record of the members of the team.
- Good and highly synergistic interactions with other teams from the department

Weaknesses

The chemotherapy sensitivity of the mouse model appears at this moment too high to allow meaningful extrapolations. Moreover the number of analyzed biopsies cannot allow drawing substantial conclusions related to macrophage function in the control of tumor development.

By taking into account the fact that the field of research on the role of PD-1 and TIL is highly competitive, it seems reasonable to redefine a precise experimental approach to assess the efficient PD-1/PD-L1 interaction potentially involved in the regulation of tumour development.

Recommendations

This team carries a real potential and has already made good progress.

In order to strengthen the competitiveness of the team and fully exploit the melanoma mouse model, the team must optimize the tools to be used to definitively ascertain the in vivo contribution of macrophages in the tumour growth. For example, specific in vivo macrophage depletion at the desired time point in the disease process would be greatly needed. This should then allow the team to go forward in patients. The observation of an activating effect of KIR is original and should be validated using primary cells in in vitro studies.

Despite the competence of all new members of the team, it would be reasonable to focus on a limited number of aims rather than developing many demanding projects. In that respect, the assessment of the role of IL-15 might not be considered as a priority.

Nom de l'équipe : Physiopathology of melanoma, combined chemo- and immuno-therapy

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	A	A

Team 30 : Viral Infection and Cytokines

This small group is currently located at the Pasteur Institute and is headed by a senior researcher with a "Pasteur Group leader" position. If the team is created at the Cochin Institute, the team leader will apply for an INSERM/CNRS position and the team will be reinforced by a senior researcher in 2010 (PU-PH). The team also includes 1 post-doctoral fellow, 3 PhD students, and a research assistant. The basic research project includes two major arms: 1) evaluation of the role of IL-7 on T-cell homing and homeostasis, leading to potential therapeutic impact; 2) characterization of Type I IFN responses during viral infection and their role on T-cell homeostasis. In addition a multicentric trial of therapeutic vaccine based on long HPV16-derived peptides patented by the group is proposed. Even if this first clinical trial has not yet begun, the team plans to develop new vaccine strategies using IL-7 as an immunological adjuvant. The line of research on IL-7 seems very promising and is further supported by a strong industrial collaboration (Cytheris SA).

Strengths :

- Good leadership of this new and dynamic team
- Solid and original project with convincing recent data, in particular in the field of the biological actions of IL-7 and their potential therapeutic relevance
- Fruitful links with the industry
- Collaborations with other teams of the Cochin Institute are already planed

Weaknesses :

The synergy between the basic and the clinical parts of the project into a global program could be improved.

Some aspects of the Type I IFN project, in particular the study of all human and simian IFN- isoforms in normal versus HIV/SIV conditions, appear challenging with respect to the small size of the team.

Recommendations :

The committee suggests to this new, and still quite small, team to focus its work and resources on fewer well-defined scientific projects.

Nom de l'équipe : Viral Infection and Cytokines

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	NN	A

Team 31 : Inflammatory diseases and immune system

This team created in 2004 is headed by a clinician with a specific and recognized expertise in spondylarthropathies, and completed by a DR INSERM and a CR1. Another DR INSERM, formerly in another INSERM unit in Necker Hospital and with demonstrated expertise in genetics of MHC and autoimmunity, recently joined the team.

The director of research who recently joined the team focuses his research on the genetic analysis of the MHC in Myasthenia Gravis (MG) and identified one SNP in the CHRNA1 gene promoter that modifies a DNA-binding motif for interferon-regulatory factor (IRF)-8 and exerts a major regulatory effect on CHRNA1 expression in conjunction with AIRE involved in immune tolerance. The clinician heading the team has mainly studied spondylarthritis and HLA-B27 associated diseases with access to the B27 transgenic rat model. In this model, he showed that CD4 T cells express TNF-a and IL-17, and he plans to assess the functional role of dendritic cells and myeloid suppressor cells. He has access to a large DNA bank from families of patients with spondylarthritis, collected over the last 10 years for the purpose of these studies, including 275 multiplex families, 70 simplex families, 900 patients, and 1650 unaffected relatives. With this material, his team has identified 2 new susceptibility loci, SPA2, SPA3. At last, the team is developing genomic approach to identify new targets in RA as clusterin and FADD as well as in Sjögren syndrome, identify therapeutic biomarkers of response to biotherapies. Thus it is proposed to analyze the molecular signature of refractory RA by using a transcriptomic and SNP microarray approaches, and to establish the role and the regulatory mechanisms of clusterin and FADD in synovitis.

The project can be summarized in 3 main aims:

1. Identification of genetic and environmental factors predisposing to SPA.
2. search of non-HLA MHC loci in autoimmune MG and SPA
3. Genomics Analysis of RA to Identification of Molecular Targets.

Strengths :

- High quality project in particular on SPA with important contribution on understanding role of B27 using rat transgenic model and genetic approach in cohorts of SPA patients.
- Members of the team have made recent breakthroughs in the field of MG and SPA.
- Good interactions between clinicians and scientists.
- The recent integration of the geneticist from Necker will clearly reinforce the strength of the team in genetics in general and more specifically regarding the role of the HLA region.

Weakness :

The project on RA seems more heterogeneous, with multiple collaborative projects using genomic platform. It would be advantageous to give priority only to few well-defined lines of this large topic of research.

Recommendations :

The committee suggests to focus and to set more manpower on the mechanistic aspects of immune response in HLA-B27-associated diseases. Studies on the immune synapse using oligomeric B27, CD4 T cells, antigen processing and molecular mechanisms should be analyzed in more depth using expertise available in the Cochin Institute.

Nom de l'équipe : Inflammatory diseases and immune system

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	A	A

Team 32 : From hematopoietic stem cells to platelet production

This team started four years ago from the merge of two previously independent groups within the hematology department. The team now consists of two senior researchers (DR2-Inserm, PU-PH), three post-doctoral fellows, one PhD student, two research assistants and a master student.

The first/last authorship publication record of the group since 2004 is quantitatively modest but includes good impact publications (JCI, J Immunol) with the senior researcher as last author. However, some excellent publications (Nat Genet, Blood, FASEB) and others relate to collaborations in which involvement of the group leader is less preponderant. The senior researcher was recently invited for lectures in the two main international congresses of this field (ASH & ISTH). Collaborations are ongoing with diverse groups in France and outside (including a submitted FP7 consortium). The group does not collaborate with scientists inside the Cochin Institute while there could be a stimulatory scientific exchange with members working on a similar field. The two main research themes in this group were TPO signaling (STATs) and HSC fate and megakaryopoiesis under physiological and pathological conditions with the extremely interesting and novel observation that cytochrome C can modulate platelet production via apoptosis.

A third senior researcher from U790, Villejuif (CR1-CNRS) will now join this group. She has expertise in HSC physiology and megakaryocyte differentiation in relation to Notch signaling (8 publications during the last 4 years).

The broad goals of the current and future research projects of this team are to study (i) regulatory pathways (Notch and STAT5) involved in HSC quiescence, (ii) the role of STATs in HSP growth and survival in normal and leukemic cells and (iii) the role of endothelium in platelet production in normal conditions and during hemopathies.

Strengths :

The study on the role of the endothelium in megakaryopoeisis and platelet production is a highly innovative aspect. The study of in vitro megakaryopoiesis under shear conditions has not been performed to our knowledge in other Labs, and could indeed be more relevant than the widely used static in vitro culture systems. The more of endothelium in megakaryopoiesis is not well known and could lead to important novel information regarding the complex process of platelet formation and production.

The last project is of excellent future potential and it is highly recommended to continue with this strategy which is internationally well appreciated and considered as relevant in the field (presented at ASH scientific session, 2008).

Solid track record

Weaknesses :

The project as a whole seems a bit unfocused and it is unclear whether the CR1 researcher who joined the team will add a new project or whether she will reinforce one of the two main projects,

Recommendations :

Some synergy inside the projects will improve the chance for success. It therefore might be interesting to primarily focus on STAT signaling in HSC quiescence and proliferation under normal and pathological conditions instead of expanding this extensive study with a novel additional pathway such as Notch.

Nom de l'équipe : From hematopoietic stem cells to platelet production

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	NN	A

Team 33 : Expansion and transdifferentiation of human stem cells

This team includes 4 researchers (1 INSERM, 1 CNRS, and 2 HU), 1 post-doctoral fellow, 2 PhD students, and 3 research assistants (2 INSERM, 1 CNRS). The research program is structured in two unrelated projects: 1) the study and clinical application of *ex-vivo* HOX-mediated expansion of human hematopoietic stem cells (HSC), and 2) the study of the myogenic conversion, including myocardiocyte differentiation, of non-muscle stem cells from different origins (HSC, endothelial progenitors, mesenchymal stem cells…) by forced expression of muscle master genes.

Strengths :

The initial observation of the potential of passively transferred exogenous HOXB4 protein to promote HSC expansion was very original and opened promising clinical opportunities. This initial report was published in 2003 in *Nature Medicine* and was heavily quoted since.

Weaknesses :

- The described system seems so straightforward that the committee is wondering why so little progress has been made since the initial report published in 2003 in *Nature Medicine*.
- Regulatory agencies and industrial partners have not been associated to this translational approach whereas it is a prerequisite to start any clinical trial.
- The second project on myogenic conversion of non-muscular cells seems immature and not connected with any of the French leaders of this competitive field.
- Very few first/last author publications in the 2004-2008 period and two scientists from this group seem to publish rarely.

Recommendations :

For potential clinical applications, more pre-clinical and clinical results are needed. Moreover, regulatory agencies and industrial partners should be associated to this translational approach. If more basic science is the main objective of the team, the committee encourages them to deal with less descriptive and more mechanistic aspects including animal studies.

Given the weakness of some parts of the scientific project and the presence of non-publishing researchers, the committee recommends that the team focuses on the propitious HOXB4 project and tries to build a rigorous and well-defined strategy to either bring soon this observation to the clinic and/or to investigate further the fundamental mechanisms underlying this intriguing finding.

Nom de l'équipe : Expansion and transdifferentiation of human stem cells

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
B	B	B	B	B

Team 34 : Antigen presentation by dendritic cells

This team includes 1 researcher (DR CNRS), 3 post-doctoral fellows, 2 PhD students, and 1 research assistant (IE CNRS).

This is a well-balanced small/medium sized team that has been conducting very high quality work on the antigen presentation pathways in dendritic cells (DCs) and on the role of DCs in the pathogenesis of HIV infection.

The research program is structured in 3 inter-related projects: 1) the study of the different DC populations (and their predictive value) in the course of HIV infection, 2) the study, mostly by immunohistochemistry, of the different areas of the secondary lymphoid organs of patients with or without HIV infection, and 3) analyze the different antigen presentation pathways in pDCs and cDCs, including cross-presentation of live cell-derived antigens, and study in vivo using mouse models whether they promote immunity or tolerance.

Strengths :

- In spite of its relative small size, this is a dynamic team carrying out excellent research.
- The group has a very good publication track record.
- The atmosphere within the team is healthy and enjoyable. This team spirit is also perceived in the networks to which the team leader contributes.
- The team presented strong and streamlined lines of research.
- The clinical work on HIV is of high relevance, well funded, and well published.
- Several strong collaborations with leading scientists in their respective field have been developed.
- The post-doctoral fellows bode well for the vitality of the group.

Weaknesses :

The study of the secondary lymphoid organs of patients with or without HIV infection appears so far mostly descriptive and correlative.

Recommendations :

The studies on the microanatomy of the human lymphoid tissues should be accompanied by functional analyses. The obvious interactions with other teams of the department working on HIV and/or type 1 interferons should be strengthened.

Nom de l'équipe : Antigen presentation by dendritic cells

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A+	A+	A+	A	A

Team 35 : Signalling pathways and apoptosis in normal and pathological erythropoiesis

This is a large team of about 20 members including 8 researchers and one research assistant (IR INSERM).

The research focus of this group is normal and pathological erythropoiesis. The group has a long standing track record of excellent work on signal transduction pathways controlled by the erythopoietin receptor (EpoR) and a recent focus has been mechanisms that control the down-regulation of EpoRs. The group is also looking at the regulation of apoptosis in the erythroid lineage of patients with early stage myelodysplastic syndromes (MDS). There is also a large body of work on signalling pathways in acute myeloid leukaemia (AML) cells. Here one important observation is that in approximately 50% of 200 patients with AML constitutive activation of the Phosphatidylinositol 3 kinase signalling pathways was detected – at least in terms of phosphorylation of the serine/threonine target Akt. This constitutive activation of PI3K was associated with better prognosis for the patients. The group has also made an important discovery that in primary AML cells, the serine/ threonine kinase Pim-2 plays an important role in substituting for mTOR signalling to control the phosphorylation of 4E-BP1 and to control protein translation.

Future projects will focus on identification of a kinase that phosphorylates a binding site for -Trcp on the EpoR. The rationale for these experiments is to understand more about the mechanisms that control the turnover of this receptor. The group has also identified an association between the EPOR and a nutrient receptor and wishes to explore the functional relevance of this association. Further work on the role of Pim-2 in AML is proposed as is a project to explore the role of Foxo transcription factors in AML. There are also plans to continue to work on the mechanisms leading to early MDS dyserythropoiesis.

Strengths :

- Overall the group presented a large body of past work with a very good balance between basic science and translational research.
- The clinically based work is relevant and highly commendable.
- The group came across as being highly cohesive with an important and focused area of research.
- The group has a good publication track record.
- There have been good recruitments to the group, which bodes well for the future vitality of the group.
- The plans for the future include a balance of quite high risk basic research (e.g the search for the EpoR kinase will be challenging) with solid efforts to translate what is known about signalling pathways that control normal lymphocytes and myeloid cells into analysis of haematopoietic malignancies.
- This is a very healthy group carrying out excellent research.

Recommendations :

Although highly relevant by themselves, it could be useful to link the projects on MDS and on AML. The future work on the role of Pim-2 in AML opens possibilities for synergies with the team 40.

Nom de l'équipe : Signalling pathways and apoptosis in normal and pathological erythropoiesis

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	A	A+

Team 36 : Type I Interferons and immune responses

The research team is composed of one group leader (CR1 INSERM) and three post-doctoral fellows. In 2006, the team received two prestigious grants: an INSERM AVENIR and a Marie Curie Excellence Team.

During the last four years the team has focused on two main axes of research: the adjuvant properties of type I interferons (IFNI) on antibody responses and on CD8+ T cell responses, and the study of memory CD8+ T cells generated by cross-priming.

The first line of research is based on previous observations made by the team leader showing that, when co-administrated with soluble proteins, IFNI have a strong adjuvant property not only on antibody responses but also on CD8+ T cell responses generated by cross-priming.

A first goal of the team was to define which TLR ligand could have adjuvant properties for CD8+ T cells. Their results (published in 2004) showed that all the tested PAMPs were able to induce CD8+ T cell cross-priming through IFNI pathway. A second goal was to define the role of DC subsets in CD8+ T cell cross-priming. The use of RelB deficient mice (lacking both double negative and CD4+ DC subsets) showed that in these mice CD8+ DCs were not sufficient to induce an efficient CD8+ T cell cross-priming (*J. Immunol 2006*). A third goal was to investigate the potential direct effect of IFNI on T and B lymphocytes (*J. Immunol 2006*). By using experimental models in which T or B cells were selectively deficient for the IFNI receptor, the team showed that the expression of IFNI receptor on T or B cells was necessary for optimal antibody responses. They also showed that IFNI R expression on T cells was required for the induction of optimal CD8+ T cell responses generated by cross-priming.

The second line of research focused on the study of CD8+ memory T cells generated by cross-priming following immunization with soluble proteins. This topic is relevant for the design of vaccines based on pathogen-derived proteins instead of live vaccines. Following immunization with ovalbumin either soluble (together with IFNI, TLR ligands or anti-CD40 stimulating antibodies) or expressed as recombinant protein in vaccinia or Listeria Monocytogenes, the efficiency of vaccination with soluble proteins in the induction of CD8+ T cell memory was evaluated. Using tetramer staining, IFN Elispot and in vivo CTL assay the team showed that CD8 memory T cells can indeed be generated by cross-priming using immunization with soluble proteins.

Based on their previous results, the team proposes to dissect in more detail the cellular and molecular mechanisms involved the generation of optimal memory responses against soluble antigens. Four main topics will be studied:

1. Further functional assessment of memory CD8+ T cell response generated by cross-priming as compared to that induced following infection.
2. Role of the anatomical localization of antigen on the development of memory CD8+ T cells.
3. Functional characterization of the DC subsets implicated in the generation of long lasting CD8+ T cell responses
4. Contribution of the tissue origin of DCs in conferring homing properties to memory CD8+ T cells

Strengths :

During the last four years, the team leader has acquired a recognized competence in the field of CD8+ memory T cells.

Some promising experimental approaches have been developed to define the role of IFNI in the initiation of CD8+ memory T cells.

The team leader has obtained important funding within the last three years.

The questions addressed are interesting and the future results on the generation efficient memory CD8+ T cell responses might be relevant to optimize vaccination strategies.

Weaknesses

Although the publication scores of the team leader as first author are very good, the team has not published regular articles during the two years.

The oral presentation and the discussion made during the site visit did not give the impression that the recently obtained results are ripe for submission.

The future of the team depends on several uncertain parameters: the possibility to recruit an additional scientist, and the possibility to obtain further funding as the AVENIR contract was not renewed and the Marie Curie Excellence grant will end by January 2010.

Finally the team leader will have to obtain the HDR to be allowed to train PhD students.

Recommendations

Concerning the results recently obtained, the team should attempt to finalize and submit a first set of experiments on the characterization of CD8+ memory T cells following immunization with soluble antigens. At this stage, it would be very important to have some publications in order to apply for grants to strengthen the team.

Among the different complementary aims proposed, it would be suitable to operate a choice. Two main topics appear very interesting and potentially successful: the impact of antigen localization on the development of subsequent memory CD8+ T cells, and the role of DC tissue origin in influencing the migratory behavior of memory CD8+ T cells.

It would seem reasonable that this team considers joining temporarily another team from the Cochin Institute having a more stable structure. This would allow the team leader to have more time to focus on her projects and would help her to generate a productive independent line of research, a prerequisite for the future development of her own team.

Nom de l'équipe : Type I Interferons and immune responses

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
B	B	B	NN	B

Team 37 : Regulation of peripheral T cell autoreactivity

This is a new team that joined the Cochin Institute in 2007. The team is headed by a DR2 CNRS and was reinforced in 2008 by the arrival of a CR1 INSERM, one young INSERM scientist, 1 post-doctoral fellow and 2 PhD students. This emerging group has already shown its ability to rapidly structure itself in a *bona fide* team.

The team, dedicated to the study of regulatory T cells, has previously demonstrated that minimal autoreactivity is required for the generation of the T cell compartment in the thymus and for its maintenance in the periphery.

The project based on cellular immunology studies in animal models can be divided in 3 major aims:

- Aim 1 is dedicated to the study of lymphopenia as a trigger for autoimmunity. Lymphopenia has been correlated with an increased onset of autoimmune disorders in humans. The project proposes to assess the frequency of CD4+ T cells undergoing spontaneous proliferation in a lymphopenic environment by injecting purified CFSE-labelled CD4+ T cells into lymphopenic hosts (CD3e-/- mice).
- Aim 2 focuses on CD8 T cells tolerance. CD8+ T cells from chimeras will be purified and injected into lymphopenic recipients, either alone or together with CD8+ T cells from normal C57BL/6 mice, in order to assess CD8+ T cell tolerance.
- Aim 3 evaluates regulatory T cells in cancer. To this end, a melanoma model, the MT Ret mouse, will be used. In this model, the RET oncogene is introduced as a transgene leading to of spontaneous melanoma development that recapitulates the natural history of human melanoma. This model will serve to investigate the role of regulatory T cells in the tumor microenvironment and in secondary lymphoid organs.

Strengths :

- High quality projects and well-structured program.
- Original projects in the very competitive field of regulatory T cells, providing a niche to this emerging team.
- Possible synergistic interactions with other scientists in the Cochin Institute.
- Although the projects are dedicated to basic immunology, they have clear clinical relevance.
- The recent integration of a senior scientist will contribute to the quality of the project.
- The publication track is decent.

Weaknesses :

- No clear links with the society and the economic world have been presented.
- The interactions with other French or international scientific partners are minimal.
- This good cellular immunology team could become more molecular within the Cochin Institute environment.

Recommendations :

The committee suggests that the team takes full advantage of the expertise available at the Cochin Institute to investigate the molecular mechanisms and the in vivo imaging correlates of their interesting findings. In particular, there is good opportunity to investigate the signalling of regulatory T cells induction. Contact with clinicians to correlate the results obtained in the murine models with those from lymphopenic patients should be undertaken.

Nom de l'équipe : Regulation of peripheral T cell autoreactivity

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	B	A	A

Team 38 : Immunopathology of hepatitis C virus

This team, created in 2006, is headed by a clinician (S. Pol) with a strong and recognized expertise in HCV, hepatitis therapy, and liver fibrosis. The team was joined by a CR1 INSERM back from the NIH in 2008 and by another member with expertise in HCV infection.

The team has shown in the last years that inactivation of liver necrosis and inflammation results in cirrhosis reversal. They have validated the FIB-4 test to score fibrosis in HCV or HBV chronic infections before treatment. Moreover, the clinical part of the team has been involved in several phase I or II trials to evaluate the safety (side effect-related discontinuation) and the efficacy of HCV protease and polymerase inhibitors. The clinical part of the team has contributed to publications with high impact in 2007-8.

The project has 3 major aims :

- Aim 1 relates to the study of the immunopathology of HCV. To this goal, the team proposes to assess viral spreading in real time in human liver slices. For this, a plasmid encoding a tetra-cys tagged HCV FL genome will be used to infect liver slices and spreading will be assessed using laser-scanning confocal microscopy. Immune cell migration in the HCV-infected liver will be studied by live video imaging. An additional and original research program relates to the analysis of the impact of genetic markers on fibrosis progression and non-responsiveness the current anti-HVC therapies.
- Aim 2 addresses the interference of HCV with antigen processing. This will be done by comparison of primary hepatocytes infected or not by HCV.

- Aim 3 will investigate the activation of fibrogenic cells by HCV infected hepatocytes by developing a two-compartment co-culture system in transwell experiments (between HCV-infected hepatocytes and stromal or immune cells).

Strengths:

- Innovative projects that may lead to major breakthrough in the field.
- The project is supported by strong interactions between clinicians and scientists.
- The clinical team has a very strong and recognized expertise in the field, and proven access to large and well defined cohorts of HCV-infected patients.
- The team proposes the use of innovative imaging technology available at the Cochin Institute.

Weaknesses :

Gap in publication of the two scientists who joined the team in 2008.

The plasmid luciferase system produces viral-like particles that may not be reflective of the diversity of the highly replicative normal HCV.

Several high risk projects in a very competitive field.

Recommendations :

The committee suggests focusing on the projects described in Aim 1. An alternative strategy should be discussed if the visualisation of plasmid tetra-cys tagged HCV FL genome expressing cells does not work on the liver slices.

As the team is still in the maturation phase, it should rapidly recruit PhD students and post-doctoral fellows, and strengthen its interactions with neighbouring teams with expertise on the immunopathology of HCV.

Nom de l'équipe : Immunopathology of hepatitis C virus

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A+	B	B

Team 39 : MAP Kinases, stem cells and hematopoietic differentiation

This group has built up a good reputation in clarifying the downstream TPO/ERK signalling pathway during HSC renewal and megakaryopoiesis. This approach has led to identification of IEX1 as a novel specific TPO-regulated ERK substrate. The head of the team is a senior researcher with a DR2 INSERM position and is an expert in the MAP pathway of TPO signalling (invited talk at one Gordon conference Meeting on megakaryocytes & platelet biology in 2005). This team also includes two other researchers with respectively a DR2 and CR1 position, one post-doctoral fellow, two PhD students and one research assistant. The researchers of this team have a good record of senior-authorship publications during the last four years (Blood, JBC, EMBO J), and are involved in teaching (master programs) and tutoring (4 PhD defences in the last four years) activities at the Cochin Institute. One researcher, who joined this team two years ago, will retire at the end of 2010.

Current and future efforts are focused on 3 major aims

- Aim 1: activation and roles of ERK isoforms in HSCs and TPO functions,
- Aim 2: identification of ERK downstream targets involved in TPO signalling under normal conditions,
- Aim 3: ERK and its downstream targets in pathological hematopoiesis (MDS, AML; T-ALL).

Strengths:

- Strong scientific track record.
- Highly original and focussed projects.
- The team leader is strongly committed to perform in depth analyses of the previous findings of the team.
- The team has a number of collaborations with other French and foreign academic groups but, surprisingly, not with the industry.
- The team is well funded by various sources.
- As a result of the above, the perspectives of this team appear excellent and feasible.

Weaknesses :

- Little interactions with other scientists working in the same field within the Cochin Institute.
- Some rather tense relationship within the team could be perceived.

Recommendations :

Given the good expertise of this group in TPO/ERK signalling, this fundamental (aims 1 & 2) and interestingly in the future also more translational (aim 3) research project is a logical extension of earlier efforts. Collaboration involving 'the in vitro megakaryopoiesis/platelet production model' within the department would be beneficial to both teams.

The study of non-TPO dependent ERK signalling in clinical samples will be performed in collaboration with other team members of the department. This part of the project could however be improved by focusing on a few key questions concerning the role ERK signalling in pathology. Focusing the described activities would likely increase the impact on the scientific community.

Nom de l'équipe : MAP Kinases, stem cells and hematopoietic differentiation

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	NN	A

Team 40 : Lymphocyte activation and immunological synapse

This is an outstanding group performing cutting edge research on T lymphocyte activation. In the last 5 years the group has made some seminal discoveries including the discovery of antigen independent contacts between T cells and dendritic cells that play a role in sensitization of antigen receptor signalling. Here the discovery of the role of transient elevations of cAMP in early T cell signalling is important. Equally important is the discovery that the Phosphatidylinositol 3 kinase signalling pathway plays a key role in controlling T cell homing to secondary lymphoid tissue via control of the expression of lymph node homing receptors such as L-selectin and the Sphingosine-1 phosphate receptor. The group has also developed a number of innovative techniques including a FRET-based system to measure the activity of the tyrosine kinase ZAP-70 in live T cells and systems using lymph node or tumour slices to allow the analysis of T cell motility and T cell interactions with dendritic cells/ tumour cells in a physiological 3D environment. The future work program will use these elegant models to probe the role of chemokines as costimulatory signals for T cells and to probe the molecular basis for the anergy often found in human tumour-infiltrating lymphocytes. Work on the molecular basis for T cell adhesion will continue as will work on the role of the Foxo family transcription factors in T cells. The group has carried out microarray analysis of Foxo-1 regulated genes in T cells and will focus on the role of one of these in T cell activation.

Strengths :

- The past and future work is exciting and truly original and innovative.
- The group has an excellent publication record and is highly internationally competitive in its research.
- The group is highly cohesive and has a focused area of research.
- The proposed work includes study of human T cells while making good use of mouse models where appropriate.
- There are good interactions with other teams in the Institute and excellent research collaborations outside the Institute.
- The team has a good mixture of senior and junior members and it is pleasing to see the individuality of the younger members of the teams be allowed to flourish.

Recommendations :

This team is an asset to the Institute and should be fully supported.

Nom de l'équipe : Lymphocyte activation and immunological synapse

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A+	A+	A+	A+	A+

Team 42 : Neutrophils and Vasculitis

This team is the result of a new association of two preformed teams: an Inserm team directed by DR2 Inserm entitled "Neutrophils : pathophysiological implications in vasculitis" created in January 2007 within the "Research Center of Necker: Growth and Signalling" (Inserm U845) and a "Research Group on Systemic and Autoimmune Diseases", UPRES EA 4058, created in March 2006 at the University Paris Descartes.

During the last four years, these two research groups have obtained significant data that justify a true association to investigate and elucidate new molecules derived from neutrophils and mainly involved in the pathophysiology of vasculitides with anti-neutrophil cytoplasmic antibodies (ANCA). Indeed INSERM team has been investigating the biology of proteinase 3 (PR3), a major target of anti-neutrophil cytoplasmic antibodies (ANCA) in Wegener's granulomatosis. By focusing on the molecular and structural study of membrane PR3, they i) characterized new PR3 targets proteins involved in the modulation of inflammation via the cleavage of anti-inflammatory proteins (i.e annexin-1) or proteins involved in the balance survival/apoptosis (i.e pro-caspase-3 or p21/waf); ii) investigated apoptosis-induced PR3 expression and its association with phospholipids scramblase 1. Moreover, the relationships between PR3 gene polymorphisms and clinical expression of vasculitis were successfully investigated. The EA4058 has been mainly involved in the identification of new autoantibodies and their specific target antigens in patients with vascular diseases including vasculitides, systemic sclerosis (SSc) and pulmonary arterial hypertension (PAH). Interestingly, IgG reactivities directed toward endothelial cells have been identified in patients with microscopic polyangiitis. Moreover, by using a new proteomic approach, this group identified in patients with idiopathic PAH and SSc-related PAH, specific target antigens of anti-fibroblast antibodies that play key roles in cell biology and maintenance of homeostasis.

Based on these key scientific results, the lead scientists have decided to reinforce the synergy between the two groups by investigating more precisely the cellular and immunological aspects of the pathophysiology of vasculitides and by taking opportunity of the very specific recruitment of patients with systemic vasculitis and biological collections of the "National reference center for systemic vasculitidis and systemic sclerosis" at Cochin hospital. Thus, the multidisciplinary and integrative project will pursue three aims: 1) the elucidation of the molecular mechanisms of neutrophil activation and the specific role of PR3 and its biological partners in the triggering of a specific ANCA-associated vasculitis, Wegener's granulomatosis; 2) the characterization of the mechanisms leading to endothelial cell activation and vessel wall injury, with a specific focus on A20, an NF-B

inhibitor, and 3) the identification of target antigens and potential pathogenic role of autoantibodies directed toward endothelial cells and vascular smooth muscle cells.

Strengths:

- This new team is a joint venture between two previous teams that have already collaborated. The association appears logical, synergistic, and highly promising.
- This synergy should be fully exploited as part of the project aims to better define the biological function of PR3.
- Members of the team have access to a unique clinical material and possess the required know-how to achieve their goals.
- The scientific track record of the individual members of the team is very good.

Weaknesses:

The merge of the 2 groups, and the addition of the junior INSERM scientist to this emerging team, should lead to an even more integrated and more focussed work plan.

The number of publications is already impressive as it is. There is no need to duplicate a number of them in the publication list of the written document…

Recommendations:

The line of research aiming at investigating the role of A20 in the regulation of endothelial cell apoptosis seems rather less promising and may need to be redefined. The molecular and functional studies of the PR3 antigen could benefit of the use of a recombinant molecule. The functional properties of the new autoantibodies identified in SSc and PAH should be analyzed using both in vitro cellular models and appropriate in vivo animal models.

Nom de l'équipe : Neutrophils and Vasculitis

Note de l'équipe	Qualité scientifique et production	Rayonnement et attractivité, intégration dans l'environnement	Stratégie, gouvernance et vie du laboratoire	Appréciation du projet
A	A	A	A	A

UNIVERSITÉ
PARIS DESCARTES

Le Président
Axel KAHN

Paris, le 17 avril 2009

DRED 09/n° 173

Monsieur Pierre GLORIEUX
Directeur de la section des unités de l'AERES
20 rue Vivienne
75002 PARIS

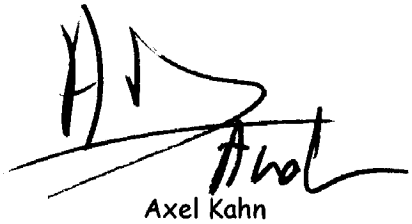
Monsieur le Directeur,

Je vous remercie pour l'envoi du rapport du comité de visite concernant l'Institut Cochin « **Immunologie hématologie** » rattaché à mon établissement.

Ce rapport n'appelle pas de commentaire particulier de la part de l'Université.

Je vous prie de croire, Monsieur le Directeur, à l'expression de ma meilleure considération.

Le Président de l'Université



Axel Kahn

Université Paris Descartes 12, rue de l'Ecole de Médecine 75270 Paris Cedex 06
Tél. 33(0) 1 40 46 16 01 Fax 33(0)1 40 46 16 43
Email : axel.kahn@parisdescartes.fr

Institut Cochin
Membre de l'IFR Alfred Jost

Pierre-Olivier Couraud
Directeur

22 rue Méchain 75014 Paris
tel.01 40 51 64 57
fax 01 40 51 64 73
u567@inserm.fr

http://www.cochin.inserm.fr

Paris, le 10 avril 2009

Réponses au rapport du comité d'experts

Report from the visiting committee

Research unit :

~~Department of Immunology~~

of the Cochin Institute

University Paris 5

The research unit :

Name of the research unit :

Requested label : **UMR CNRS, UMR_S INSERM**

N° in case of renewal :

Head of the research unit : **M. Pierre Olivier COURAUD**

Head of the Department: **Mr Alain TRAUTMAN**

University or school :

Université Paris 5

Other institutions and research organization:

INSERM

CNRS

Dates of the visit :

December 8-10, 2008

Team 29: Physiopathology of melanoma, combined chemo- and immuno-therapy

The team was reorganized with the arrival of a young experienced **researcher** recruited as CDD INSERM (3-5 years) and a senior researcher, DR2 INSERM; both significantly broaden the expertise of the team in terms of anti-tumor immunity and biology of NK cells.
The validation of the activating effect of KIR with ITIM in primary cells is a major issue that we will address. The project on IL-15 will not be pursued further after the description of the membrane IL15 in renal cell, as described in a recent manuscript published early 2009:
Khawam K, Giron-Michel J, Gu Y, Perier A, Giuliani M, Caignard A, Devocelle A, Ferrini S, Fabbi M, Charpentier B, Ludwig A, Chouaib S, Azzarone B, Eid P. Human renal cancer cells express a novel membrane-bound interleukin-15 that induces, in response to the soluble interleukin-15 receptor alpha chain, epithelial-to-mesenchymal transition. Cancer Res. 2009 Feb 15; 69(4):1561-9.

Team 30: Viral Infection and Cytokines

Reply to the committee

I would like to thank the AERES committee members for their advises concerning the project we presented for the creation of a new research team in the Institut Cochin.

As pointed out by the committee, I am presently *chef de laboratoire* at the Institut Pasteur and, with the strong support of the direction of Institut Cochin, will postulate for an INSERM and/or CNRS DR position in 2010. For the next 2 years, the direction of the Institut Pasteur allowed me to be "*détaché avec salaire*" at the Institut Cochin.

We do agree with the committee that the clinical trial concerning HPV therapeutic vaccine is a bit on the side of our main projects. However, this funded and close to start trial is the accomplishment of a long lasting research program initiated several years ago by the clinician (PU-PH) who joined the team. Moreover, our knowledge on HPV peptide immunogenicity will help evaluating the potential of IL-7 as an adjuvant for mucosal immune responses upon vaccination. This part of the project is in direct continuity with the work we performed over the last few years demonstrating the major role of IL-7 in mucosa-specific T cell homing (Beq et al. 2009, Blood *in press*).

Our close and long lasting collaboration with Cytheris S.A. not only concerns IL-7 but also interferon alpha subtypes project. Indeed, a PhD student is presently funded through a CIFRE convention with this company on the IFNα project. Thus, all the biochemical part of this project (production and purification of the IFNα isoforms) will be performed by the company. We will then use the purified proteins to evaluate their therapeutic potential in culture. Finally, the few promising IFNα

subtypes, as determined by their *in vitro* activities, will be tested *in vivo* in SIV-infected macaques for their anti-viral activity and their limited immunomodulating function. This project is supported by the ANRS (AO 2009-1) for a 3 years period.

Finally, while our team is presently composed of 2 senior scientists, 1 research assistant, 1 post doc and 3 PhD students, it will be, upon creation, expanded to a total of 10-12 persons through the recruitment of a former post doc in the lab who will postulate for a position at INSERM/CNRS, and several post-doc fellows. Moreover, we will pursue our long lasting and fruitful collaboration with the group "Antigen presentation by dendritic cells" (Nascimbeni et al. 2009, Blood *in press*) and develop new collaborations with various teams at the Institut Cochin, especially the groups ""Immunopathology of hepatitis C virus" and "Neutrophils and vasculitis".. We believe that our group will be strong enough to achieve the goals of both the IL-7 and IFNα projects.

Team 31 : Inflammatory diseases and immune system

Please note that the group is co-headed by a PU-PH and a DR Inserm, who are closely collaborating, especially on the development of the spondylarthritis (SPA) research axis.

Response:
We thank the Evaluation Committee for the positive comments and the constructive criticisms.
We would like to emphasize that to a large extent the recommendations of the committee match quite well the strategic orientation that we have already adopted over the last 4 years, and proposed to reinforce in the future. Indeed, both leaders of the group are now strongly involved in pursuing the work on SPA and have jointly re-organized the team with a research activity dominated by genomics combined with functional validation of candidates genes identified by genetic or genomic studies. In this regard, it is noteworthy that only 3 people were involved in SPA in 2004 compared to 11 today. The recent reinforcement of the team with the arrival of a group led by a widely recognized immunogeneticist (DR1 Inserm) goes also straightforward along this line. That is why we particularly appreciate that the Committee stressed the several strengths of our group that are in direct agreement with this effort. Conversely, we would like to moderate their perception that some other aspects of the research developed by the group, notably on RA, should be considered as a weakness. This part of our project is currently funded through several competitive grants (ANR, Ligue Contre le Cancer, Société Française de Rhumatologie, Foundation Arthritis) and led recently to 3 PhD theses (including two by rheumatologists recently promoted as PU-PH) and the publication of several significant papers (PNAS, J. Immunol, JBC, ..). Most importantly, it allows highly fruitful collaborative studies with numerous clinicians nationwide and the development of technical skills (transcriptomics, pharmacogenomics), all of which are directly relevant for the SPA main project, whether for cohorts' recruitment or for in-depth investigation of candidate

targets. Finally, and as mentioned in our report and underlined during the presentation, we have already prioritized well-defined lines, in particular transcriptomics of synoviocytes, as we fully agree that this field is quite large.
Regarding their recommendation to develop more research focused on the immunobiology of HLA-B27 and its implication in immune synapse between antigen presenting cells and T cells, we would like to stress that these questions have already been highlighted in our project, as shown by funding through a dedicated ANR program (acronym: DCSPA), which involves existing collaborations with other groups on site and two recent papers (Arth Rheum, 2007, 2008).

Team 32 : From hematopoietic stem cells to platelet production

The AERES committee got the feeling that "our research project was a bit unfocused with the coming senior researcher possibly adding a new project in parallel with the other members of the team" (point 1). In addition, your committee mentioned that "our group does not collaborate with scientists inside the Cochin Institute" (point 2).

Point 1, The senior scientist who recently joined the team got expertise on each of the two main projects of the team and will actually reinforce each of these, instead of bringing a new theme:

- She has focused her research on the endothelial specific Notch ligand Dll4, which she recently found regulating platelet production as well (manuscript submitted); such a regulator should thus participate to the crucial endothelium/megakaryocytes interactions that we (Dunois et al, in revision to Blood; 2008 ASH oral presentation) have just recently demonstrated. Her researches will thus focus on the endothelium/Mk interactions, determining the role of endothelial Dll4 on platelet-forming Mk and on platelet production under thrombogenic stimuli or high shear stress. These researches have been just submitted to ANR Emergence bio and ARC.
- She has developed expertise on the *in vivo* assays that assess stemness of population of cells enriched in human or murine hematopoietic stem cells (Lahmar et al, Stem cells, 2008). This expertise will now allow the team to study the biological activities of hematopoietic stem progenitor cells made deficient for Stat5a or 5b factors (PhD thesis already submitted to Paris7 University). Her interest on Dll4 led her to observe that Dll4 fulfils regulatory activities on stem cell quiescence/self-renewal in addition to its role on endothelial cell biology and platelet production. She has identified the RNA-regulators Pumilio factors as Dll4/Notch target genes, which, interestingly, have just been independently characterised as HoxB4 targets by the team 33 of our Institute. Such a convergence, associated with our expertise on the cytokine TPO and its impact on translation regulatory processes (Caron et al, Mol Cell Biol, 2004), will help both

teams in deciphering post-transcriptional processes involved in regulating maintenance of hematopoietic stem cells.

Point 2, we forgot mentioning clearly our strong collaborations with members of our department :
. Team 33: (Rouyez et al, J Immuno, 2005; Morison et al, Nature Genetics, 2008; Dunois et al, Blood under revision), and also Dr A. Dubart-Kuppershmitt'team who just left (Boukour et al, J Thromb Hemost, 2006). We have recently intensified our collaboration with this team by designing complementary strategies to decipher Pumilio actions in hematopoietic stem cells (see above), involving one post-doc team 33 (since March 2009), and one student from our team (since January 2009). - In addition, we also have initiated collaborations with Team 22, because of strong convergence in the regulators of human mesenchymal/muscle and hematopoietic stem cell quiescence. We have just set up a collaborative research program on Stat factors, which has been submitted for financial supports at the AFM (March 2009); Part of this program has also been included in a PhD program submitted to Paris 5/7 Universities.

Team 33 : Expansion and transdifferentiation of human stem cells

Answers to critics of the AERES committee.

1) Expansion of hematopoietic stem cells is a very competitive topic that needs original and highly developed results to be published in highly ranked reviews. In the last few years, apart from several large-public publications, we contributed to the redaction of a book (Handbook of Cell-Penetrating Peptides) and 2 publications were achieved in 2007 and 2008 (Haddad *et al*, Leukemia; Haddad *et al*, Stem Cells). Moreover, a report including important results (presented orally during the evaluation) that direct further works of the team will be submitted for publication soon.

2) Translational approaches for clinical use of HOXB4 are currently developed by a part-time member of the team (MD, PhD) at the Clinical Investigation Center (CIC) of Institut Gustave Roussy (IGR). Clinical trials must be performed at the IGR and industrial partnership will be established by the CIC. Our specific contribution will consist in experimental *in vitro* and mouse trials.

3) Concerning the myogenic conversion of non-muscle cells, our work has progressed and 2 papers are in preparation. We foresee that a third one can be submitted within 4 months. These studies are developed in collaboration with several French teams, in particular:
- team : *Régénération, pathologies et thérapies du muscle squelettique humain,* Myology Institute (Pitié-Salpétrière Hospital, Paris);

- team : *Développement et pathologie du Tissu Musculaire*, INRA-UMR703, Veterinary School of Nantes;
- team : *Excitabilité cardiaque normale et pathologique*, CNRS-UMR8162, Le Plessis Robinson;
- team 25 : *Génétique, développement et physiologie des muscles striés* (Cochin Institute).

Key *in vivo* studies are currently developed in collaboration with several of these teams. In the near future, in line with the committee's recommendation, we plan to limit this activity to collaborative epigenetic studies on myogenic conversion of non-muscle stem cells.

4) The two researchers whose activity is questioned are, in fact, in charge of students and responsible for the main projects of the team (both HOXB4 and muscle projects). They had to adapt their previous research orientations to these projects. Finally, they will sign as last authors the papers in progress.

5) Our projects are supported by a series of recent grants, which clearly confirm that our results and projects are widely recognized and supported by the scientific community :

- 3-year labeling by the Ligue Nationale Contre le Cancer (2009-2011) (210 K€),
- 2-year grant of the Association de Recherche contre le Cancer (2009-2010) (50 K€),
- Grant from the Association Française contre les Myopathies, (27 K€),
- A 2-year post-doctoral grant from the Fondation pour la Recherche Médicale has just been attributed to a post-doc of our team.

Moreover, we applied for the attribution of 2 additional collaborative grants:

. a INCa-DHOS grant, in collaboration with the *CIC Biothérapies* of IGR: intention letter accepted (110 K€ requested for our team),

. an ANR grant, in collaboration with the *Laboratoire de biologie cellulaire hématopoïétique*, Inserm U718, Institut Universitaire d'Hématologie, St Louis Hospital, Paris (83 K€ requested for our team).

Team 38 : Immunopathology of hepatitis C virus

Reply to the committee

We believe that our project to study real time viral spreading in human liver slices is very original; at the same time, we acknowledge the risk taken given the competition in the field. The good news is that our preliminary results have demonstrated that sustained infection of the slices is possible; the amount of

infectious materials released in the supernatants is of the same magnitude as that obtained with primary hepatocytes. We therefore put a lot of confidence in this project that is now becoming our main focus.
We just recruited a chinese Ph.D. student to work on it (three-year grant from CNRS).

Team 42 : Neutrophils and Vasculitis

Answer from the team 42: Neutrophils and Vasculitis

1) The committee suggests that the three main scientists involved in the project (the co-directors and the junior Inserm) should work on a "more integrated and more focused work plan". Since January 2009, we developed our projects in agreement with this view with 2 main axes: the first is the molecular and functional study of PR3 and the second is the molecular mechanisms involved in endothelial damages in ANCA-associated vasculitis. This latter subject will include the investigation of the role of the autoantibodies identified by one of us and also the study of A20 as a potential modulator of endothelial damage. In that way, the A20 project is totally merged into the main project and is a potential interesting track rather than an independent project by itself. Moreover, since January 2009, we are jointly working to set up cellular models of endothelial cells and vascular smooth muscle cells to study the functions of the autoantibodies: these are ongoing experiments. Animal models will be developed next according to results obtained with in vitro systems. For instance, in pulmonary hypertension project, this will be done in collaboration with a group at the *Centre Hospitalier Marie Lanelongue* who has all the expertise in experimental models of pulmonary arterial hypertension.

2) The committee suggests to use "recombinant molecule" for the study of PR3. PR3 cannot be expressed as an active proteinase in bacteria, yeast or baculovirus system. This possibility has already been investigated by several groups and especially by one of us (FEBS letter 1996; Protein expression purif 1996). Therefore, in order to be fully active, PR3 has to be purified from mature human neutrophils. This is a limitation for producing PR3 in high amounts for epitope mapping. In our project, we are using recombinant PR3 as well as mutant PR3, which are expressed in myeloid cells (mast cell lines) which is the only system to express active PR3 and to investigate the function and the structure of PR3.

Since the presentation, 4 original research articles and one review article have been accepted for publication:

S. Moriceau, C. Kantari, J. Mocek, N. Davezac, J. Gabillet, I. Chiara Guerrera, F. Brouillard, D. Tondelier, I. Sermet-Gaudelus, C. Danel, G. Lenoir, S. Daniel, A. Edelman and V. Witko-Sarsat. Coronin-1 is associated with neutrophil survival and

is cleaved during apoptosis: Potential implication in neutrophils from cystic fibrosis patients. *J Immunol* 2009 in press

Servettaz A, Nicco C, Goulvestre C, Guilpain P, Cherreau C, Vuiblet V, Guillevin L, Weill B, Mouthon L, Batteux F. The different forms of human systemic sclerosis reproduced in mice by specific pro-oxidative agents. 2009. *J Immunol* (*sous presse*).

Bussone G, Dib H, Dimitrov JD, Camoin L, Broussard C, Tamas N, Guillevin L, Kaveri SV, Mouthon L. Identification of target antigens of self-reactive IgG in intravenous immunoglobulin preparations. *Proteomics* 2009 Mar 19. (Epub ahead of print)

Terrier B, Tamby MC, Camoin L, Guilpain P, Bérezné A, Tamas N, Broussard C, Hotellier F, Humbert M, Simonneau G, Guillevin L, Mouthon L. Anti-fibroblast antibodies from systemic sclerosis patients bind to α-enolase and are associated with interstitial lung disease. *Ann Rheum Dis* 2009 Mar 16. (Epub ahead of print).

V. Witko-Sarsat, S. Daniel, L.H. Noël and L. Mouthon. Neutrophils and B-cells in vasculitis *APMIS* (review) 2009 in press

Pierre-Olivier Couraud
Directeur de l'Institut Cochin