Discussion following Dr. Gallo's Talk

Dr. Hunt: Dr. Gallo has been reviewing the properties of reverse transcriptase and also informed us about recent experiments to produce antibodies against it. Before we come to the second point, can I ask about one property of this enzyme, its capacity to make double-stranded DNA. What is known about it? Does it in fact make double-stranded DNA?

Dr. Gallo: In most of our experiments the endogenous reaction has been done in the presence of actinomycin D. We, therefore, have not adequately looked at this. However, Peter Duesberg has.

Dr. Duesberg: Yes, they do, but you know we do not know much about this synthesis of DNA other than short pieces of DNA are made perhaps 5 or 6 S in size given a template of 3 or 4 million daltons at least. In the primary reaction it makes complementary DNA, and then the secondary reaction leads to double-stranded DNA also of small size. Whether this is the whole story, that is the complete transcription from RNA that ultimately leads to a complement of double-stranded DNA, as it presumably happens in the cell, is completely open.

Dr. Gallagher: I would like to go back to your (Dr. Gallo) hypothesis that you drew with regard to a hot spot in the DNA because if that is true you might predict a new form of virus and this could get out and infect other cells and so forth. You might be able to test this. Spiegelman's lab could perhaps tell us if there is an increase in the differences between normal and leukemic DNA over a period of time in a leukemic patient, perhaps during the course of CML or something like that. Have they checked it?

Dr. Hehlmann: No, we don't have sequential data in one patient through the course of the disease except one case of ALL, in which we detected viral related RNA during the acute phase of the disease that was not detectable after remission. (Spiegelman and his group have completed these results. There are no leukemic DNA sequences in leucocytes during remission. Moreover, leukemic DNA sequences have been found in the leukocytes of only the leukemic member of identical twins; Proc. Nat Acad. Sci., 70, 269–2632).

Dr. Kufe: At first, according to this hypothesis you said that you think that the malignant state requires the addition of exogenous information and then you went on to evolve the hot spot hypothesis and proposed it was a mutation or addition via recombination of that hot spot. Now is that saying that there was oncogenic potential in that sequence that just had to be altered. Is this just a variation of the oncogene hypothesis only that it requires a base change or something like that?

Dr. Gallo: I believe Dr. Kufe wants to know if the proposed hot spot is either just a sequence that is specially receiving a carcinogenic "boost" thus being just a variant of the oncogene theory, somatic mutation, or added new information? The proposal

demands new information. There was only oncogenic potential by virtue of its unusual susceptability to change. This is clearly distinct from the oncogene hypothesis. However, regarding the nature of the change, I don't think it is useful to attempt to distinguish between the alternatives since as yet the data available, including the important paper that your lab published in this respect, might be explained by amplification, i. e. a difference in some nucleotide sequences between normal and leukemic cells, sequence X after transformation becomes X 50. Your experiment may not differentiate between those two possiblities. Moreover, it is of course, not yet proven that those "extra" sequences are pertinent to leukemogenesis, although I would like to assume with you that they are.

Dr. Kufe: I have to answer that according to the sensitivity of these assays, it would be impossible to have X originally to be amplified. That is, X had to be introduced from the outside because we would have picked up X on the hybridization assay.

Dr. Gallo: Are there viral (type-C RNA tumor vires) genes in some normal cells? Everyone by now must believe that there are some virogenes in at least some normal cells. I would like to know where they came from – or which came first – are these virogenes in fact really cell genes which the virus utilizes? Duesberg should speculate on this.

Dr. Duesberg: That's too much for me. That's like all theories on the origin of life: Where do whales come from, where does God come from, where does a "clean chicken" come from? But I would like to return a question to you, may be somewhat related to that. I think we can at least divide those viruses which cause cancer and those which are sub-virus like things which may be a consequence of cancer. I think that those which are causing cancer may be like the men and the other more or less like the boys. So I think that shouldn't be confused too much. I think these sub-viral particles or endogenous viruses or incomplete endogenous viruses or enzymes might in fact well be a consequence of cancer rather than its cause. But I think there is little doubt that Rous SV or AMV can be the cause of cancer.

Dr. Gallo: I kind of agree with that, at least they cause chicken cancer. I think the information for carcinogenesis may be packaged into only very special type-C RNA tumor viruses. But I wouldn't even make those viruses that you call boys any less important because boys can become men. Moreover, we have now demonstrated that the reverse transcriptase in human leukemic cells and the viral related nucleic acid is related not to endogenous non-oncogenic type-C viruses, but specifically to type-C viruses which in fact *are* oncogenic such as the woolly monkey simian sarcoma virus.

Dr. Duesberg: That is absolutely right. I could have called them girls but I gave you boys. Maybe I could ask one more question. When you talk about leukemia or certain types of myeloblasts that you clinically find are these all genetically or antigenically homogenous? In a given type of luekemia, is there always a unique population of cells? Or could there be heterogeneity, could it be a random thing, just a random messing up of differentiation? Or could it be that it all results from a single cell and

leukemic cells are all identical? I think the identity of leukemic cells would be more compatible with a genetic or viral theory whereas "random" could be regulation or who knows what?

Dr. Stohlman: There are a restricted number on types of leukemia and frequently one sees a monotonous type of cells morphologically. I don't know that anyone has analysed the genetic information from these to say it is identical from cell to cell.

Dr. Duesberg: In these chromosome linked diseases like the Philadelphia chromosome, do you see the change only in the leukemic cells or also in other cells?

Dr. Stohlman: The erythroid (red) cells and the megakaryocytes (platelet precursors) all have the same chromosome.

Dr. Hehlmann: I now refer to Dr. Gallo's very interesting data on antibodies prepared against reverse transcriptases of primate viruses which cross react well with your human leukemic enzyme and offer a new immunologic approach. You have just said you have not prepared an antibody against your human leukemic reverse transcriptase. You had that enzyme fairly purified in the past. What are the difficulties in producing an antibody?

Dr. Gallo: We have been giving it to Bob Nowinski, and he is inoculating rats with the pure enzymes. So far we are losing a lot of enzyme, i. e. no antibody to date. I am becoming very discouraged unless we come in with about a 1 000 grams of leukemic cells so that we can get a lot of this enzyme and are then able to hand him one or two milligrams of enzyme. We tried only twice, we failed, and we didn't relish the idea of losing more of this enzyme.

Dr. Stohlman: I would like to ask Bob one question which shows my immunological incompetence, is it necessary to really clean up and purify this enzyme. Don't some people suggest that you have a better chance of forming good antibodies if things are dirtied up a bit and then you absorb the sera later?

Dr. Gallo: I don't know that I am more competent than you are in immunology but I will answer as best I can. In the first trials when the antibodies to viral reverse transcriptase were prepared, the reverse transcriptase wasn't purified enough and it is true, success was achieved easier in those laboratories which didn't purify as much. It is also apparently true that when you purify more, you reduce antigenic potency. However, if you finally succeed with the purest preparation you are obviously in a much better position. In the long run the results as well as the antigen are cleaner.

Dr. Hofschneider: I have to apologize if I don't ask about reverse transcriptase and such things. I would like to come back to the colony stimulating factor. I have just met, maybe one or two weeks ago, some cell biologists and have asked them for the factor and they told me it is better to forget about it as a specific agent. Apparently, here in the audience are many people who believe in this factor. I would like to have some more information. Is it known what is the chemical nature of the factor, has it

been enriched and to what extent, and has it been applied to animals and what was the effect?

Dr. Stohlman: It's a glycoprotein and various molecular species have been reported from 15.000 to 60.000. It has been given to normal animals. There is a problem in giving it to normal animals in that it is difficult in the experiments done thus far so separate effects of the "release factor", the release of granulocytes from the storage compartment, from true proliferation. The studies to date just don't separate them. I can't answer the question of its physiologic role. When human marrow is cultured with CSF after 12–14 days you get a significant number of eosinophilic colonies, maybe 30, 40, 50 percent, and in the normal human being you certainly don't see this degree of eosinophilic myelopoiesis. So I would raise the question if maybe CSF is a triggering substance, there being other regulators. Most of the evidence suggesting a physiologic role for CSF is inferential. I'm sure it does have one but for various reasons I don't think we have worked it out.

Dr. Torelli: Since I have been under provocation by Dr. Gallo to give my views I would like to say something about the nature of the leukemic cell. I think it is quite evident that we are talking about the etiology of leukemia and we're talking about the virus which is probably being brought into the leukemic cells but we still have to deal with the question, what are the leukemia cells. Because it is quite clear that we are trying to get rid of this question simply by saying: well, these cells do not mature, these cells are blocked in maturation. I think that we should be carefully comparing normal immature cells with leukemic cells. It's quite true that for a long time, in attempting to compare leukemic cells with normal cells, studies were hampered by the fact that many were comparing cells which were proliferating (leukemic) and cells which were not proliferating (normal). I think results at this stage of our studies were useless. We are really faced now with one main question. What is the key difference in the leukemic cell and the appropriate normal cell control. I think that point has to do with the introduction of a viral genome into the cell. This introduction should not bring the cells to limited progression. I think we should look for major changes which are brought into the cell by the introduction of the virogene.

Dr. Gallo: There is one point which I don't think came up at any time in the meeting. I am referring to some cases of bone marrow transplatation. There were two reported cases of recipients that were leukemic who received bone marrow from normal donors and apparently when relapses occured the normal donor cells had transformed in the recipient patient. Now there's a lot of discussion of how valid those observations were; how clear were the results which were based on cytogenetics. If, however, these results are valid, there is obviously a very important lead which directs us to almost only one conclusion, that a transforming agent remains in at least some leukemic patients. We can talk about cell-cell fusion, but I doubt whether this would occur under these conditions *in vivo*. Even *in vitro* under the best conditions, it is difficult. Normal donor cells then apparently can transform in recipient leukemia people, and the most likely interpretation is that the "transforming factor" is still present after destruction of leukemic cells.

Remarks Concerning the Discussion

It was quite impossible to include in this book the whole discussion, which lasted for more than 12 hours, in its entirety. We had to, unfortunately, leave out the greater part, and limit the discussion to the summary reviews of Dr. Fred Stohlman, a clinical hematologist, and Dr. Robert Gallo, a medical molecular biologist. Even from the discussion following Dr. Stohlman's and Dr. Gallo's reviews we were forced to cut a great many interesting critical remarks, and were only able to include 30 to 40 per cent. For the critical selection we thank Drs. R. Hehlmann and T. Hunt.

Because of space limitation many of the authors have included a considerable part of the discussion in their articles and many questions, arising from the different investigational trends, have been summarized in the introduction.

Rolf Neth