Immunogenetics of Cancer and Aging

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INTRODUCTION

Thank you for taking time to visit my poster.

This is a summary of views about immunology and disease, particularly cancer, going back to the early days of cellular immunology and before the characteristics of the T-cell receptor or even the existence of the HIV virus were known. The program abstract allowed too few words, so here by introduction is the expanded version.

Antigen altered idiotype immune regulation explains resistance to viruses, cancer, autoimmune disease, and AIDS: Implications for treatment.

ABSTRACT

Role natural killer cell activity in HIV infection, aging and breast cancer survival. Natural killer cells participate in the elimination of tumors and virus infections. Data presented here include their role in a virus infection (HIV), metastatic breast cancer and aging per se. We have analyzed two gene segments (MHC HLA class I ligands and NK receptors), and each with single amino acid differences, that together can identify long survivors. Homozygosity of HLA–Bw4 supertype in HIV progression has been described. Since HLA-KIR genetic interactions of HLA-Bw4 with KIR (3DS1) only included Bw4, with the exception of B*27 and B*44 that use different mechanism of protection on the clinical outcome of HIV infection; NK–ligand interactions (NKG2A-HLA-E with HLA class I leader peptides) to explain the long term non-progressors (LTNP) to AIDS. HLA-B alleles have Methionine (Met) or Threonine (Thr) (at second position (P2) of their leader peptide.HLA-Bw4 alleles, with exception of B*38, encode leader peptide, with Thr at P2 and HLA-Bw6 encode Met at P2 are B*07, B*08 and B*14,all others have p2 Thr. Comparisons of the Thr in LTNP with progressors up to 15 years with non-infected Caucasian controls were significant. Also, aged Mexicans demonstrated significant increase of homozygosity of p2 Thr and Mexican patients with metastatic mammary cancer were studied after 10 years of treatment with surgery and chemotherapy; 39 survivors demonstrated significant increase of homozygosity of the p2 Thr compared to controls without cancer and 37 non survivors.

DISCUSSION

Antigen altered idiotype (AAI) is fundamental to the immune response and regulation of antigen specific immune responses. When the specific immune response phenotype segregates in a Medelian fashion this phenotype or trait has been called an immune response gene (IR-X). The most thoroughly characterized IR-genes are alterations of polymorphic portions of major histocompatibility complex (MHC) molecules. For delayed type hypersensitivity (DTH) and other Th1 responses and for Th2 responses like CTLs and T-suppressor/regulatory cells, the actual targets have been well characterized. The antigenic determinants of proteins and other combinations of amino acids like GAT, are usually 8-10 amino acid peptides forming a complementary alteration to a portion of the MHC molecule. Chemicals like DNP can directly alter the polymorphic yet specific idiotype of the MHC itself. During development an individual's repertoire of antigenic responses evolves in the thymus gland. Because strongly anti-self and therefore potentially damaging clones must be eliminated or specifically suppressed, a large portion of the antigenic repertoire cross reacts with an allogeneic histocompatibility complex. Singer and Williams (Cell Immunol 4:1-4, 1978) proposed that such cross reactivity would eventually overlap with important responses like those to opportunistic infectious organisms, and thereby result in a serious hole in the repertoire that could cause an acquired immunodeficiency syndrome, now known as AIDS. Logically, the changes resulting from exposure to multiple incompatible allogeneic MHC molecules could produce the opposite effect, namely an increase in a desirable immune response.

The allogeneic effect could enhance the response to tumors, viruses, etc. Benacerraf et. al. named the allogeneic effect as a mechanism for increasing T cell help for antibody responses and resistance to transplanted leukemia in guinea pigs. Williams et. al. showed that such tumor resistance could be localized to the mouse MHC (Cancer Res 35; 1586-90, 1975). A few years ago a male patient with HIV developed AML. The fact that his leukemia was later controlled and possibly even cured with chemotherapy and a mixed unrelated stem cell transplant makes our case. The patient, who had a significant HIV titer before the transplant, to date has not shown any evidence of HIV infection (Blood). This case and the abundance of support for the graft versus tumor effect in leukemia and lymphoma patients given allogeneic stem cells are examples of the allogeneic effect in people. The altered idiotype hypothesis also to supports Duesberg's speculation that HIV is not the immediate proximate cause of AIDS (Nature 1995 May 18;375). The virus certainly infects and kills some lymphocytes but we propose also that HIV is a relatively unique and very potent inducer of altered idiotype immune suppression. In strict genetic terms, syndromes like AIDS or survival with cancer are phenotypes, i.e. traits, as an expression of genotype or DNA sequences. Immune response genes, such as IR-GAT or IR-EAE etc., and by analogy IR-AIDS or IR-Cancer Survival, should be viewed the same way. IR-something that is "genetic" demands a more precise explanation of mechanism. This was done for IR-GAT and other antigens by Benacerraf (Nobel Lecture, 1980) and later in more exquisite detail by Zinkernagel and Doherty (Nobel Lecture, 1986).

DISCUSSION (cont.)

Jerne actually initiated the idiotype network concept (Nobel Lecture 1984). It was our attempt to relate his theory to the MHC and immune responses that led to the antigen altered idiotype hypothesis. F1 hybrid mice are resistant to histocompatible tumors, the resistance is MHC (H-2 in mice, HLA in man) dependent and improved via the allogeneic effect. Freireich's group has demonstrated the same resistance to AML using transfusions of allogeneic lymphocytes (McCredie et. al. Current problems in cancer. Vol. 3. Chicago: Year Book Medical Publishers, Inc.; 1978:4-37). Manipulation of immune responses with allogenic transfusions could influence resistance to cancer and autoimmune diseases. To quote Dausset (Nobel Lecture 1980) "This deeper understanding of man's immune response will quickly have major repercussions in pathology. It will perhaps provide the key to the irritating problem of the treatment of cancer..."

The term "idiotype" evolved in immunology after what Oudin called "idiotypy" after the Greek which means unique. He was addressing the phenomenon that rabbit antibodies against an antigen (Ag) Salmonella typhi could themselves become specific antigens for another rabbit to produce an antibody that would specifically bind only to anti-Salmonella typhi antibodies. Thus it would be an anti-anti-salmonella (Ag) antibody. The anti-anti-Ag concept was expanded in depth by Jerne into what became the network paradigm for how the immune system is specifically generated and regulated. That anti-anti-Ag antibodies could be auto-anti-anti-Ag, i.e. produced by the same individual, was eventually demonstrated (Rodkey LS J Exp Med 139:712, 1974).

Fundamental at every step was then, and still is now, the necessary role of the antigen. However, after two decades of intensive study (see Green MI, Nissonof A eds. The Biology of Idiotypes, Plenum Press, New York, 1984) the idiotype paradigm came to a more or less ignominious demise (see Eichmann K, The Network Collective: Rise and Fall of a Scientific Paradigm, Birkhauser Press, Basel).

RMW entered the field forty years ago from the perspective of Mendelian genetics, cellular immunology focusing on thymus-derived cells, autoimmune disease, and particularly tumor immunology. We defined idiotype in the original context, something unique, and likely genetic, determined by very polymorphic or highly mutated genes, like MHC or immunoglobulin variable regions. Our laboratory was trying, unsuccessfully, to reproduce and then expand studies suggesting that T-cell dependent response to antigen altered idiotype could turn off immunity to various antigens. The most impressive observation to us was that T-cell immunity to MHC antigens in the presence of the antigen, could turn off transplantation immunity, presumably anti MHC (Binz H, Wigzell H Cold Spring Harb Symp Quant Biol 41: 275-284, 1977). Studies of immunological tolerance, even to small molecules like DNP, always required the antigen to be present in the system, usually at high concentrations. DNP specific immunological tolerance is a good example (Borel Y, Transplan Rev 31:3, 1976). We speculated that the key target was idiotype (Singer DS and Williams RM (1978) Cell Immunol 1) and that alteration of this unique structure by antigen would perturb, dysregulate, and certainly suppress immunity.

Because polymorphic MHC was clearly involved in virtually every genetically controlled antigen specific immune response (IR-genes), we presumed that MHC structures must be participating in specific immune suppression as well. The fact that specific tolerance was T-cell dependent, led to the speculation that suppressor T-cells might well inactivate responding T or B cells in a specific manner. In the case of autoimmune disease, it might be possible to turn it of by T-cell immunity to autoantigen altered idiotype. We tried unsuccessfully to do this with an MHC dependent genetically controlled autoimmune diseases; EAE with basic protein antigen and responder type (IR-EAE positive) rat MHC (Lewis). We did find, that in radiation chimeras, it was required that the MHC of the thymus in which the T-cells matured contain the Lewis (IR-EAE responder) genotype. This coincided with a similar requirement for MHC linked CTLs in the LCM system (Zinkernagel RM et. al. J Exp Med 148:805-810, 1978) but there was no support for turning off the EAE generating cells by autologous CTLs. In the mouse model of EAE several laboratories have done elegant experiments that support our hypothesis.

For immunity to MHC molecules one of the most recent observations consistent with the hypothesis that antigen altered idiotype induces immune suppression were the elegant experiments reported recently by Kapp and her colleagues (J Immunol 179:2105-2114, 2007). T suppressor cells are now called regulatory T cells or just Tregs and the function is dependent on the transcription factor FoxP3 which drives naïve T cells into suppressive/regulatory mode, depend on TGF beta, and express CD25 the alpha portion of the IL-2 receptor.

Moreover, Tregs require direct cell contact, including of course the antigen, to exert a maximal effect (see for example Thornton AM and Shevach EM J Exp Med 188:287-296, 1998).

When a foreign antigen is injected repeatedly at low doses or at very high doses, specific tolerance can be generated, and this tolerance can be transferred with T-cells, not antibody. The foreign antigen does not persist indefinitely. Unless the tumor is completely eliminated by whatever means, tumor antigen (TAg) will be continuously produced, regardless of the host immune response. We opined that this is like continuous low antigen dose, or intermittent high antigen dose induced tolerance and that this could be mediated by T-suppressor cells.

DISCUSSION (cont.)

Now we would call them Tregs. We showed that resistance to P815 mastocytoma in histocompatible mice was genetically controlled and MHC dependent (Williams RM, Dorf ME, and Benacerraf B Cancer Res 35:1586-1590, 1976). The relevant MHC were located in both Class I and Class II molecules as defined by studies with H2-K and I-A mutants (Kwak L et. Al. Cancer Res 57:5754-5757, 1983). This genetic trait, MHC linked resistance to P815, was defined as "mice live longer" and that the immune response was responsible. The actual tumor antigens involved were not known but the one observation we made to support the theory that TAg altered idiotype induces immune suppression against the tumor antigen, was that Cr-51 labeled B6D2F1 lymphocytes which come from P815 tumor bearing animals can be killed in culture, only when unlabeled P815 cells are added. We speculated that these were CTLs specific for TAg altered idiotype.

We did show in radiation chimeras that the response depended on the genetics of the recipient thymus epithelium (Williams RM, Eig B and Singer DS in Genetic Control of Natural Resistance to Infection and Malignancy, Academic Press, pp 477-483, 1980).

Growing tumor is a situation where antigens, even tumor antigens, are continuously being exposed to the immune system. Cytotoxic T lymphocytes which are directed against tumor associated antigens, likely associated with MHC molecules, could not only kill tumor cells, but could also kill or suppress lymphocytes which are responding to the tumor antigen.

Specific and non-specific tumor immunity was the other focus of our laboratory until attention was turned to treating cancer in people. Nowadays the terms "adaptive and innate" immunity would be used to replace "specific and non-specific". After thirty years of treating cancer it is even clearer that if we define tumor resistance as "living longer when a cancer is present", then survival depends on genetically controlled, MHC dependent immunity. This review, and particularly the abstract for AAI, of which the author has been a member for 42 years, is a plea for basic immunologists to pay closer attention to human anti-tumor immunology in patients with known cancer. Cancer treatment research has been dominated by questions like what is the molecular basis for cancer development and growth and how can more cancer cells be eliminated from the body?

Tumor immunology has focused mostly on what is the antigen against which adaptive immunity can be generated? Most of the clinical efforts to boost non-specific or innate immunity, and thereby make patients with cancer live longer, have had very limited success.

Cancer cells can be removed by surgery, killed by radiation, heat, or cytotoxic chemotherapy. However, metastatic cancer can never be completely eliminated from the body without an immune response. Models of tumor treated with ever higher doses of chemotherapy or radiation in culture, or more commonly tumor in animals treated with frequent repeated doses of chemotherapy or radiation, never kill ALL of the tumor cells. Physicians will appreciate the analogy to microbes, antibiotics never all of the microbes.

Patients with metastatic cancer only die from the cancer when some system vital for survival is compromised beyond repair. Death from cancer, and sometimes progression free survival, has become nearly the only criterion for FDA approval of any new treatment. In Mendelian genetic terms let us define the increase in survival with treatment "X" as a trait. When we could use the term "immune response gene" or IR gene, when talking about the genetics of immune responses, the antigen used in the experiment, for example GAT is added so we called the trait of response an IR-GAT gene. These genes would now be defined as class I or class II MHC gene products, the variable regions of MHC molecules (idiotypes or epitopes or paratopes for which there are DNA sequences to determine their structure). When the antigen is not as precisely known as GAT, a random polymer of Glu Ala and Tyr, the phenomenon or trait can still be described as an IR-gene. In our case IR-EAE or IR-P815 resistance, etc.

For human studies call the trait "resistance to cancer of the blank". Resistance here does not mean resistance to developing cancer of the blank, but rather resistance to dying from or with cancer of the blank. Two examples make the point, prostate cancer and melanoma, but the concept would likely be true for cancers originating from any cell type. In prostate cancer the phenomenon of living longer can be caused by passive transfer of autologous mononuclear cells (many are dendritic cells) which have been cultured with a molecule present on prostate cancer cells genetically combined with GM-CSF. This is now known as the FDA approved treatment Provenge. The effect of living longer is seen regardless of whether any other parameters of having prostate cancer (e.g. PSA level or bone scan) actually improve. We propose that specific and/or non-specific immunity, i.e. adaptive and/or innate immunity is enhanced in a genetically determined fashion. If any of the effect is adaptive immunity involving antigens, and some has to be because the control was "naked" autologous dendritic cells, then the phenomenon will be to some extent HLA dependent. Recently a randomized trial of HLA specific prostate specific peptides showed significantly reduced progression free survival (Noguchi M et. al. Cancer Immunol Immunother 59:1001-1009, 2010).

DISCUSSION (cont.)

Breeding or even family studies to prove the role of HLA in survival with cancer are of course not feasible. HLA associations for survival with a particular cancer will have to suffice. When the original AAI hypothesis was proposed in 1976 we knew nothing about the disease know known as AIDS and the HIV virus had not been discovered. Our hypothesis focused on the antigen and immune suppression plus the relationship of the specificity repertoire to MHC antigens.

We proposed that the down side of the allogeneic effect may be involved and, to the extent that the host was exposed to multiple different MHC specificities, then the antigenic repertoire including some important common infections, e.g. pneumocystis and other opportunistic infections could overlap the "hole" in the antigenic repertoire caused by suppression of responses to HLA antigens. So called Gay Related Immune Deficiency (GRID) represented an immune suppression among individuals exposed to multiple different HLA antigens. No virus was required for our hypothesis, then the HIV virus was characterized and put our theory out of business. Duesberg speculated that while the HIV virus did infect and kill lymphocytes, it was actually the immune suppression that defined the syndrome. We speculate that both are true, the syndrome AIDS is from immune suppression and HIV happens to be particularly potent for inducing that suppression, at least in part through antigen altered immune suppression.

Substantial progress has been made in the application of innate and adaptive immunity for the actual treatment of cancer patients (see Anderson KS,

Cancer Investigation, 27:361–368, 2009 for a very readable and yet comprehensive review)

There is even one study that supports our speculation on adjuvant chemotherapy where MHC dependent adaptive immunity might even be as effective as the adjuvant chemotherapy itself (Peoples GE et. al. J Clin Oncol 23;7536-7545, 2005). Moreover, the allogeneic effect to help cancer patients live longer, as first described by Freireich's group in 1978, has actually been applied even in solid tumors (see Bishop MR et. al. J Clin Oncol 22; 3886–3892, 2004 and Lundqvist A and Childs, R J Immunother 28: 281–288, 2005).

Clinical progress will require finding drugs that significantly enhance the MHC dependent adaptive immune response. Add to these some approaches that take advantage of the positive side of the allogeneic effect, and improved survival of patients with metastatic cancer can be achieved.

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RESULTS

Experiment	Strain	*n*	*H-2*	MST(days)	*p*[a]	Survivors at 81 days
1	B10	36	*b/b*	36.5		0
	B10 x B10.BR F_1	26	*b/k*	50.8	0.0003	7
	B10 x B10.M F_1	33	*b/f*	59.1	<0.000002	15
2	B10	22	*b/b*	42.4		0
	B10 x B10.WR F_1	18	*b/ja*	64.1	0.000002	10
	B10 x A F_1	16	*b/a*	47.3	0.043	0
3	B10	25	*b/b*	31.8		0
	B10 x B10.D2 F_1	27	*b/d*	36.44	0.053	0