IMMUNOGENETICS

Hypothesis for Cancer Survival

R. Michael Williams MD PhD

Northern California Cancer Center

ABSTRACT

Immune surveillance reflects an ongoing war between the immune system and the specific cancer. If after primary treatment, the honeymoon period of "no detectable cancer" lasts a lifetime, then we say there was a cure by primary therapy. Adjuvant therapy alters the outcome statistically. Adjuvant trials are balanced for known relevant patient and tumor characteristics but NOT for the genetics of the host or the detailed genetics of the tumor. Small absolute differences seen in adjuvant trials likely reflect an imbalance in these important characteristics, and adjuvant drugs or radiation alter the immune response in addition to killing some tumor cells. In short, survival probably depends as much on the immune system as it does on the cancer treatment.

We proved that survival with a histocompatible tumor was genetic and immunological. Single amino acid differences in H-2 can change survival. Adaptive immune responses are antigen or epitope specific and almost always involve class I and class II MHC genes. T-cells are involved and the immune response is regulated so that decreasing T-suppressor cells (Tregs) or their function can result in increased survival (ipilumumab in melanoma). Immunization, even after the cancer and its antigens are in abundant supply, can prolong survival (Sipuleucel-T in prostate cancer). It is time to resurrect the term Immune Response (IR) genes, this time linking the phenomena in tumor immunology to survival (IR-S genes). We propose that the clinical oncology community investigate the Immunogenetic Hypothesis for Cancer Survival, and predict that HLA genes and T-cell immunoregulation effects will participate importantly in how long a cancer patient will survive.

INTRODUCTION

IF a man or an inbred mouse has a cancer, AND it is not because of a transplanted tumor, THEN survival time is dependent on the genetics of the tumor, the genetics of the individual or inbred strain, AND the genetics of the immune response to what must be autochthonous, i.e. self antigens (a.k.a. epitopes or idiotypes). This is the Immunogenetic Hypothesis for Cancer Survival (IHCS). At the 2011 AAI meeting in San Francisco, we presented an update of the Antigen Altered Idiotype Hypothesis first described in Cellular Immunology in 1978. The self idiotype can be altered by some not-self molecules, like virus proteins including HIV. The altered idiotype could include the antigen combining region of an immunoglobulin molecule or a polymorphic region of a histocompatibility molecule, Class I (H-2K in mice and HLA A,B,C in man) or Class II (H-2I-A and HLA-D)

The original description of H-2K and I-A polymorphisms that can influence cancer survival required using a transplanted tumor into F1 hybrid mice created between two homozygous inbred strains, one of which was parental. Because the tumor came from one of the homozygous inbred strains, there was no histoincompatibility. The survival time has to depend on the F1 host's response to the tumor. If there were no variability in technique, then all the mice would expire on the same day. A look at the survival curves shows that they were very uniform. The median or even mean survival time was the tested survival parameter because all the mice died. A Kaplan-Meir survival curve and calculation of hazard ratio was not necessary. Statistically significant differences with low p-values were usually seen. Summarized below are the typical survival times, and the genetic effects for the key observations:

RESULTS

Experiment	Strain	n	H-2	MST(days)	p[a]	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

Table 1. Survival of B10 and various F_1 hybrid animals after injection of 5000 viable fibrosarcoma cells of B10 origin [1]

The survival of B10 animals is compared to that for hybrids produced between B10 females and males with various recombinant H-2 alleles. The experiment shows that the B10 x 5R F_1 animals survived significantly longer than B10's and that B10 x 2R F_1 animals succumbed significantly sooner than B10 or any other injected group.

Experiment	Strain	n	H-2	MST(days)	p[a]
1	D2 x B10.D2 F_1	29	*d/d*	30.1 ± 1.3[b]	
	D2	42	*d/d*	26.8 ± 1.3	0.062
	D2 x B10.BR F_1	28	*d/k*	28.8 ± 1.0	0.305
	C3H x D2 F_1	24	*d/k*	28.3 ± 1.7	0.085
	D2 x B10 F_1	20	*d/b*	35.7 ± 2.4	< 0.0001
	B6D2 F_1	26	*d/b*	49.7 ± 2.5	< 0.0001
	D2 x B10.S F_1	22	*d/s*	37.6 ± 2.3	<0.0001
2	D2 x B10.D2 F_1	24	*d/d*	22.7	
	D2 x B10.BR F_1	12	*d/k*	21.8	0.537

Table 2: Survival of various DBA/2 (D2) hybrids given injections of 1000 viable P815 mastocytoma (D2 origin) cells [1]

The results demonstrate that D2 x B10F_1, B6D2F_1, and D2 x B10.SF_1 mice survived longer than the D2 x B10.D2F_1 control animals when given injections of the P815 tumor.

RESULTS

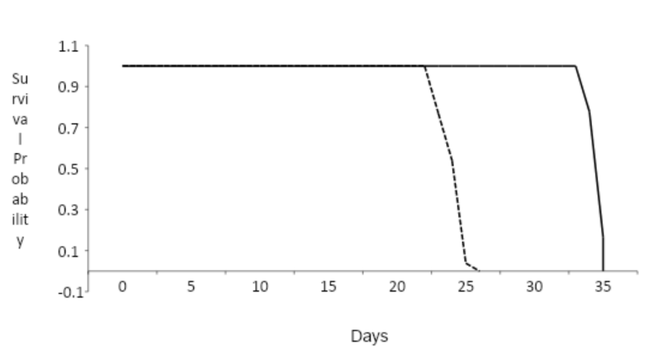

Table 3. Schematic representation of survival curves from numerous P815 survival experiments [5]

	Number Tested (F/M)	Number dead	Survival (days) Median	Survival (days) Mean	S.E	Difference from B6 P
B6	12 (0/12)	12	31	31.7	2.45	-
B6.C-H-2^{bm1}	13 (5/8)	13	32	35.7	4	0.467
B6.C-H-2^{bm11}	17 (9/8)	17	26	25.5	1.32	0.0012
B6-H-2^{bm5}	18 (7/11)	18	25	26.6	1.57	0.0282
B6-H-2^{bm3}	10 (6/4)	10	24	24.6	1.25	0.0008

Table 4. P815-X2 resistance in DBA/2J x (B6 or mutant) hybrids [3]

Changes involving as few as one or two amino acids in at least one of the class I, H-2 histocompatibility alloantigens, as well as I-A in other experiments are capable of altering resistance to otherwise histocompatible tumors. This experiment, which implicates the independent roles of H-2K and I-A genes in hybrid resistance, supports the original concept (Williams et al.1975) that more than one H-2 gene is involved in histocompatible resistance to P815-X2. I'm doing a MRI to

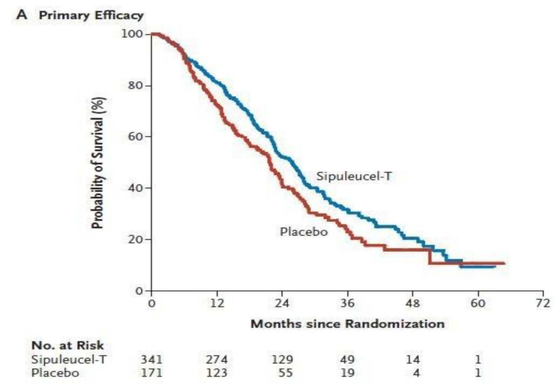

Figure 1: Kaplan-Meier Estimates of Overall Survival [6]

The panel shows the results of the primary effectiveness analysis of treatment with Sipuleucel-T as compared with placebo. This figure comes directly from one of the many published descriptions of the Sipuleucel-T (Provenge) trials. The placebo controls received the same treatment without the antigen.

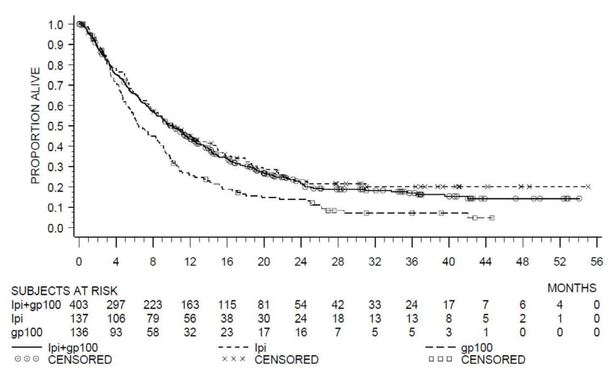

Figure 2: Overall Survival in the gp100 and ipilimumab melanoma trial [7]

The graph demonstrates the overall survival in the Ipilimumab+gp100 arm compared to that in the gp100 arm. The overall survival in the Ipilimumab+gp100 arm was also compared with the Ipilimumab arm. The ipilumumab resulted in increased survival. The gp100, whether or not ipilimumab was used, did not.

DISCUSSION AND CONCLUSION

The importance of the MHC in the IHCS may not be limited to immunological mechanisms alone. For example, H-2 and its subregions are involved in whether or not different chemicals or viruses produce cancers. In these cases it still could be that the immune surveillance mechanism was responsible, but there is some evidence to implicate other mechanisms like glucocorticoid metabolism. Likewise, it is important to keep in mind that survival per se, or the inherent limitations of lifespan for a particular species, is also genetic. The MHC is involved here also, along with what might happen to the population, e.g. an epidemic, as we showed in mice.[5]

As a corollary to the IHCS, we must define the tumor associated antigens that are involved. There are antigens (epitopes) that either are or are not tumor associated cancer survival antigens (TASAs). Ironically, this has rarely been defined in experimental animal models, but the two survival curves shown as Figures 1 and 2 here do define each type of TASA in man. For prostate cancer, prostate acid phosphatase IS a TASA, because specific immunization against it results in prolonged survival among prostate cancer patients treated with Sipuleucel-T. On the other hand, gp100 is NOT a TASA for melanoma. The well constructed trial of ipilimumab (Yervoy) has within it clear evidence that immunization against gp100 does NOT confer any cancer survival advantage for melanoma. The ipilimumab trial also represents one of the only clinical trials in which the two groups were actually balanced or stratified for any genetically polymorphic histocompatibility antigen. In this case all patients were HLA-A2 because the gp100 antigen, in association with HLA-A2 represents a good target for cytotoxic T lymphocytes. No randomized clinical trials of a drug, procedure, or radiation have ever had any stratification for the probably most important immunogenetic polymorphisms of the patients, namely HLA-A,B,C and particularly HLA-D. The small absolute differences in overall survival seen in most large randomized trials of any treatment might well be explained by an imbalance between the two groups for HLA gene polymorphisms. We have designed an experiment to test this hypothesis in breast cancer, and we suspect similar tests using already completed randomized trials that have banked tissue samples would show one or more HLA polymorphisms to be over or under represented among long term survivors.

We propose that in the case of humans with cancer, because clinical trials are never matched or balanced for the polymorphisms of HLA, many of the positive results and perhaps some false negative results could be explained by differences among the study population for relevant immune response survival (IR-S) genes. The clinical oncology community, with the help of immunologists, and geneticists, should go back to the concept of IR-genes. Define the question to investigate an" IR-some Cancer Survival", and study the immune response to whatever treatment that might help patients live longer. The experiment we have designed will test this hypothesis in breast cancer patients who are long term survivors compared to short term survivors.

REFERENCES

1. Williams, R.M., Dorf, M.E., and Benacerraf, B., 1975, H-2-linked Genetic Control of Resistance to Histocompatible Tumors, *Cancer Research*, v. 35, p. 1586-1590.

2. Singer, D.E., Williams, R. M., 1978, Hypothesis: Altered Idiotype Induces Immune Suppression, *Cellular Immunology*, v. 39, p. 1-4.

3. Williams, R.M., Kwak, W.L., and Melvold, R.W., 1981, Evidence for Involvement of the H-2K^b and I-A^b Genes in Hybrid Resistance to P815-X2, Immunogenetics, v. 13, p. 351-353.

4. L.C.J.M. Oomen, M.A., 1988, Influence of Mouse Major Histocompatibility Complex *(H-2)* on *N*-Ethyl-*N*-nitrosourea-induced Tumor Formation in Various Organs, *Cancer Research*, v. 48, p. 6634-6641

5. Gelman, R., Watson, A., Yunis, E., and Williams, R. M., 1990, Generics of Survival in Mice: Subregions of Major Histocompatibility Complex, *Genetics Society of America*, p. 167-174.

6. Kantoff, P.W., Higano, C.S., Shore, N.D., Berger, E.R., Small, E.J., Penson, D.F., Redfern, C.H., Ferrari, A.C., Dreicer, R., Sims, R.B., Xu, Y., Frohlich, M.W., and Schellhammer, P. F., 2010, Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer, *New England Journal of Medicine*, v. 363, p. 411-422.

7. U.S. Food and Drug Administration, Full Prescribing Information, 2011. Retrieved from http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/125377s0000lbl.pdf

ImmunoGenetics.com